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THE UNIVERSITY OF ALBERTA

ENDOCRINE GLUCOREGULATORY RESPONSE TO EXERCISE IN
DEPANCREATIZED ISLET CELL AUTOGRAFTED DOGS

BY

ANDREW J. PORTIS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

DEPARTMENT OF PHYSICAL EDUCATION AND SPORT STUDIES

EDMONTON, ALBERTA

FALL, 1987

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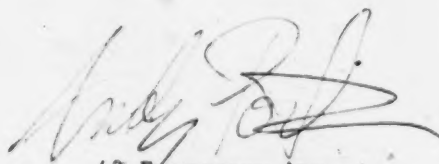
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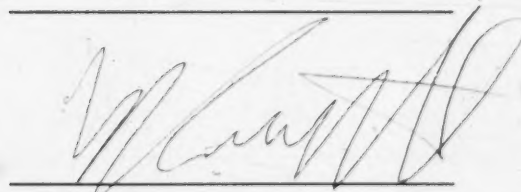
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MASTER OF SCIENCE.



Supervisor







Date: 2/10/87

ABSTRACT

Exercise in normal dogs is characterized by a dynamic regulation of insulin and glucagon secretion and the maintenance of relative euglycemia. This study examines the glucoregulatory response to exercise in totally pancreatectomized dogs, who were rendered normoglycemic in excess of one year following the splenic reflux of autografted Isolated islets of Langerhans. Venous blood was collected at 15 minute intervals during a 30 minute baseline period followed by a 60 minute run (100m/min, 12% grade). Heart rate and norepinephrine responses to exercise were equivalent in control (n=6) and transplanted (n=6) dogs. Absolute glucose concentrations were equivalent during exercise in control and transplant dogs, although the transplanted dogs exhibited a slower post-exercise recovery. Mean ($\pm$ SEM) end-exercise levels of plasma glucagon, epinephrine, and lactate were higher in transplants (405 $\pm$ 84 pg/ml, 673 $\pm$ 183 pg/ml, 3.1 $\pm$ 0.86 mmol) than controls (272 $\pm$ 40 pg/ml, 288 $\pm$ 60 pg/ml, 2.5 $\pm$ 0.32 mmol). Insulin levels were suppressed until the end of exercise in control dogs while transplanted dogs exhibited a mid-exercise surge above baseline in both insulin and c-peptide levels. A strong correlation ($r=0.81$, $p<0.001$) between glucagon and epinephrine in the transplanted dogs suggests that the exaggerated glucagon response to exercise was due to circulating β adrenergic stimulation of the autografted A cells in absence of direct neural control. The unusual insulin response observed in the transplanted dogs may have been a result of stimulation of the B cells by glucagon, or a loss of direct central control secondary to denervation. Alternatively, the exaggerated responses in both insulin

and glucagon may have arisen secondarily to splenic contraction, a normal component of the canine hemodynamic exercise response, through either direct physical ejection or indirectly through environmental changes following splenic contracture. The excessive counterregulatory response observed in the transplanted dogs was not correlated with decreases in plasma glucose, suggesting an alteration in glucoregulatory control in the long-term islet cell autografted dogs.

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I INTRODUCTION

Transplantation of isolated islets of Langerhans holds great potential as a therapy for diabetes mellitus. To date the great majority of the research in this area has been devoted to overcoming technical barriers to transplantation in humans, such as isolation yield, graft placement, and immunological aspects of transplantation (Gray and Morris 1987). These studies have achieved great strides and have brought islet cell transplantation to the brink of clinical trials. The transplantation of isolated islet cells has been observed to maintain depancreatized dogs insulin independent and normoglycemic for prolonged periods (Kneteman et al 1986, Warnock et al 1987). However, largely because studies in this area have been so strongly directed towards immediate clinical application, a full examination of physiological impact of isolation and transplantation of islet cells, with inherent denervation, has not been addressed.

The islets of Langerhans play an essential role in the control of carbohydrate metabolism. Within the islets are four different populations of endocrine cells, referred to as A, B, D, and F cells which secrete glucagon, insulin, somatostatin, and pancreatic polypeptide, respectively. Of this group, the A and B cells have received the greatest attention because of the well recognized physiological role played by their secretory products, while the D and F cells are only beginning to be understood. In spite of this attention, the control of A and B cell secretion, due to its multifactorial nature, is poorly understood.

The control of insulin and glucagon secretion can be considered to be mediated at two levels (Pipeleers 1986). At the level of the individual A or B cell there are well defined responses to changes in metabolite concentrations (Pipeleers et al 1985a, b). These metabolic stimuli have been referred to as "nutrient signals" (Pipeleers 1986) and the response is mediated by the individual islet cell. The individual islet cell is also subject to regulation from outside of the islet cell. These "neurohormonal signals" are qualitatively different from the nutrient signals. While the nutrient signals stimulate the islet cells, the response is mediated at the level of the islet. In contrast, the neurohormonal signals can be considered to dictate a response and the islet more or less passively complies.

At rest, the nutrient signals are generally considered to be adequate to control circulating glucose levels (Woods et al 1986). While the islets are quite capable of regulating their secretion to maintain euglycemia, they cannot anticipate imminent changes in substrate concentration and are not sensitive to glucose flux. The cephalic phase of the insulin response to feeding occurs before blood glucose levels are effected and is thought to play a role in preparation for the absorption of nutrients (Woods et al 1986). During stress states where an elevated glucose flux is required, the neurohormonal signals are believed to be important in controlling islet cell secretion independent of nutrient signals (Halter et al 1984).

Exercise is a physiologic stress state which requires a very high glucose flux, approximately three times higher than basal (Wasserman and Vranic 1986). Because of the high flux, any disparity between the rates of glucose production and utilization will have a magnified impact on

blood glucose levels. The glucoregulatory demand is such that control must be exerted before blood glucose concentrations change. There is a well defined islet cell response to exercise which is generally considered to be achieved through neurohormonal control of the islets. In rats (Luyckx and Lefebvre 1974, Harvey et al 1974), dogs (Vranic et al 1976, Wasserman et al 1984), and humans (Cochran et al 1966, Wahren et al 1971, Bottger et al 1972) insulin secretion is inhibited and glucagon secretion is elevated while blood glucose levels remain relatively constant. The changes in insulin and glucagon secretion have been prevented by α and β adrenergic blockade, respectively, suggesting a major role for the sympathoadrenal system in mediating these responses (Luyckx and Lefebvre 1974, Harvey et al 1974, Galbo et al 1976, Galbo et al 1977, Simonson et al 1984). Until recently, it was generally accepted that a decrease in insulin secretion and an increase in glucagon secretion could only be achieved by adrenergic mechanisms. However, in the past few years a number of neuropeptides have been localized to nerve fibers within the islets and have been observed to inhibit insulin secretion (Dunning et al 1986, Pettersson et al 1986, 1987, Tatemoto et al 1986). The discovery of a role for neuropeptides (Ahren et al 1986) and the recent re-evaluation of adrenergic stimulation (Schuit and Pipeleers 1986, Ahren et al 1987a, b) has forced researchers to reconsider previous theories regarding neural control of the endocrine pancreas.

An aspect of experimental islet cell transplantation which has to date been overlooked is the impact of transplantation on neurohormonal control of the islets of Langerhans. To examine this issue, this study examines the endocrine response to exercise in depancreatized islet cell

4

autografted dogs. Since this response occurs in normal dogs in the absence of large changes in blood glucose concentration, it is anticipated that exercise may be used as a probe of neurohormonal regulation of the graft. Although reinnervation of islet cell autografts has been reported (Madureira et al 1985), it is uncertain whether the transplanted islets would recover their full physiologic complement of cholinergic, adrenergic and peptidergic innervation. While the exercise induced changes in islet cell secretion are generally believed to be under central control and regulated by neurohormonal mechanisms, no studies have specifically addressed this issue. If the islet response to exercise is solely mediated by adrenergic mechanisms, then the impact of denervation may be limited because circulating epinephrine should be able to substitute for neurally released norepinephrine. However, the effects of splanchnic nerve stimulation on islet secretion could not be reproduced by norepinephrine infusion, suggesting that there may be a peptidergic role in what was previously considered to be an adrenergic phenomenon (Ahren et al 1987a, b).

A second, and perhaps more important, objective of this study is to carry out preliminary observations of the capacity of islet cell autografted dogs to maintain glucoregulatory control during exercise. There is currently a great deal of confusion in the literature regarding the essentiality of the pancreatic response to exercise for glucoregulatory control. Several studies have observed that a perturbation in the insulin (Kawamori and Vranic 1977, Felig and Wahren 1979, Issekutz 1980, Martin et al 1981) or glucagon (Issekutz and Vranic 1980, Wasserman et al 1984) response to exercise results in departures from euglycemia. However, recent observations by Cryer and co-workers

(Hoelzer 1986a, b, Tuttle et al 1987) during exercise in humans strongly suggest that the pancreatic and sympathoadrenal responses to exercise are redundant. The glucoregulatory response of the transplanted dogs will depend not only upon the capacity of the autografted islets to respond correctly to neurohormonal stimuli, but will require a response from the entire glucoregulatory system.

This study will yield important information regarding the potential of islet cell transplantation to truly "normalize" diabetic recipients. The capability of islet cell transplant recipients to control carbohydrate metabolism during stress states may limit the benefits of the procedure. Islet cell transplantation has the potential to relieve diabetics of their insulin dependency and prevent the complications associated with chronic poor control of blood glucose. However, if these same diabetics are unable to assume normal lifestyles because they are unable to respond to stress, they may be better off without the transplant. Therefore, the objectives of this study are to examine not only the secretory response of long-term autografted islets but, in addition, the capability of autograft recipients to maintain glucoregulatory control in the face of physiologic stress.

II METHODS

ANIMALS

Twelve adult male and female dogs of varying breeds and crosses were studied. Control dogs (weight 21.9 ± 0.83 kg, $n=6$) were obtained approximately one month before the experiment and autografted dogs (weight 21.8 ± 0.72 kg, $n=6$) were examined a minimum of one year post-transplant. All dogs were maintained on a diet of meat (360g Dr. Ballard's) and burger bits (600g Pow R Pac) with the transplants receiving coated pancreatic enzyme supplements (Cotazym, Organon Corp.). All dogs were allowed unrestricted light exercise twice daily and water ad libitum. As well, all animals were under veterinary supervision. Pancreatic fragments containing islets of Langerhans were isolated and refluxed into the splenic vein following total pancreatectomy as detailed previously (Warnock et al 1983). Briefly, pancreatic fragment isolation was performed through a modification of the Horiguchi and Merrel (1981) procedure where the pancreas was perfused with collagenase via the pancreatic duct, minced, mechanically dissociated, and filtered through a 400 μ screen. The resulting pancreatic fragment containing suspension was refluxed into the superior and inferior terminal polar splenic veins.

PRE-EXPERIMENTAL PROCEDURES

All dogs were progressively familiarized with the treadmill (Quinton Instr.) over a two week period preceding the experiment without use of noxious stimuli, as indicated in figure 1a. At the end of the

familiarization period, all dogs were subjected to a progressive intensity exercise test, modified from Ordway et al (1983), which was designed to measure maximal heart rate responses to exercise, as indicated in figure 1b. Heart rate was monitored telemetrically (Fortin Corp) and continuously displayed on a digital cardiometer (Quinton Instr.) and recorded from an ECG trace. Criteria for stopping the test were no further increases in heart rate to increases in exercise intensity, or alternately, physical inability of the dog to continue. Twenty-four hours prior to the experiment, a catheter was placed percutaneously in the external jugular vein under halothane anaesthesia. The catheter was advanced to place the tip near the right atrium and the exposed portion was sheathed in silastic tubing to prevent kinking. The catheter was then heparin locked and the area dressed.

EXPERIMENTAL PROCEDURES

The experimental protocol adhered to is presented in Figure 2. All dogs were fasted for 18 hours prior to the experiment. At the initiation of the experiment, the dead space in the catheter was withdrawn and a saline infusion (.75 ml/min) was initiated after the dog was placed in the treadmill cage to maintain catheter patency. After a 30 minute pre-exercise period to establish baselines, the dogs were exercised for 60 minutes at a treadmill speed of 100 m/min on a 12% grade, and then were observed through 30 minutes of recovery. Blood was sampled at 15 minute intervals during rest, exercise, and recovery. During sampling, the saline infusion was shunted off and the deadspace in the line, approximately 4 ml, was drawn and discarded. An 11 ml blood

sample was then drawn, the line flushed with saline (approx. 4.5 ml), and the saline infusion continued.

BLOOD ANALYSIS

The sampled blood was separated into three tubes and centrifuged immediately. For measurement of catecholamines, 5 ml of blood was placed in a tube containing EGTA and glutathione. For analysis of plasma glucose, lactate, and immunoreactive insulin, 4 ml of blood was placed in a heparinized (143 USP units) tube. For measurement of immunoreactive glucagon and c-peptide, 2 ml of blood was placed in a tube containing EDTA (0.05 ml 7.5% sol'n) and Trasylol (1000 KIU). After centrifugation the plasma was separated into appropriate tubes for storage and placed on ice, with the exception of the catecholamine sample which was placed on dry ice until the completion of the experiment. Upon completion of the experiment all tubes were stored at -80°C until analysis.

Plasma glucose concentrations were measured on a Beckman glucose analyser using the glucose oxidase method. The enzymatic reduction of NAD by plasma lactate was measured spectrophotometrically. Epinephrine and norepinephrine concentrations were determined by reverse phase high performance liquid chromatography (Hjemdahl et al 1979), after alumina extraction (Anton and Sayre 1962). Insulin was measured by a double antibody disequilibrium modification to the radioimmunoassay method of Morgan and Lazarow (1963) with a minimum limit of sensitivity of 1 μ U/ml (courtesy of R. Gingerich). C-peptide was determined by double antibody radioimmunoassay (Polonsky et al 1983) with a minimum limit of sensitivity of 0.05 pmol/ml (courtesy of K. Polonsky). Glucagon was

measured by double antibody radioimmunoassay. Variability on all hormone assays was approximately 10 percent.

STATISTICAL PROCEDURES

Delta scores were calculated by subtracting the mean of the baseline period for each dog from observed values. Statistical main effects were analysed by repeated measure ANOVA with a Greenhouse-Geiser adjustment for missing data points. Paired and unpaired T-tests were used, where appropriate, to determine significance. Correlations were calculated by Pearson product correlation. The 0.05 level was selected for statistical significance. All values are expressed as mean $\pm$ SEM unless otherwise indicated.

- A**
- Day 1. 15 min., 75m/min., 0% grade
 - Day 2. 30 min., 100 m/min., 4% grade
 - Day 3. 30 min., 100 m/min., 8% grade
 - Day 4. 30 min., 100m/min., 12% grade
 - Day 5. 45 min., 100m/min., 12% grade
 - Day 6. 60 min., 100m/min., 12% grade

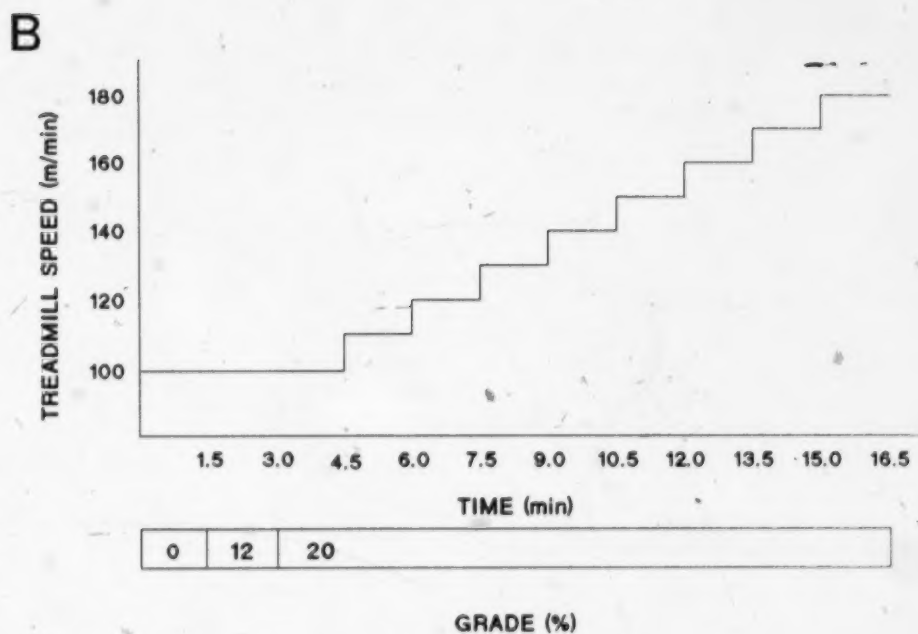


Fig. 1

a) Treadmill familiarization program b) Progressive intensity exercise test.

Test was stopped when plateau was observed in heart rate response to increase in intensity or if dog was unable to continue.

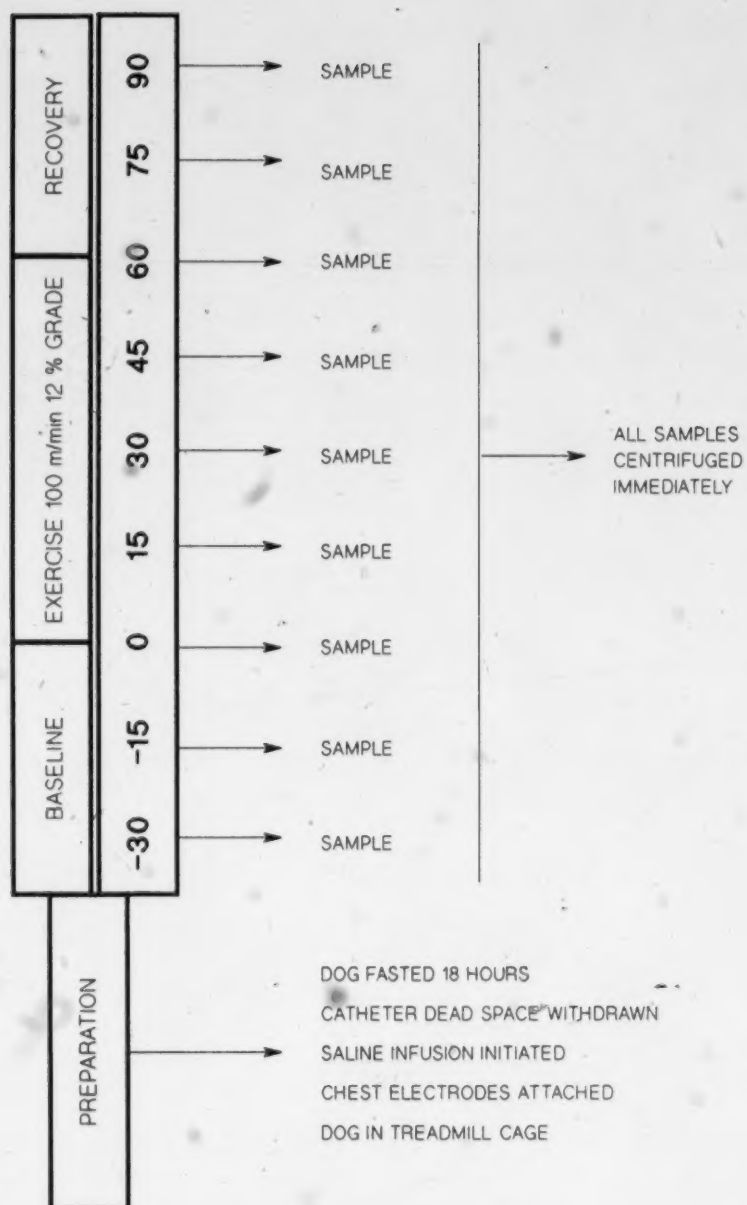


Fig. 2
Experimental protocol

III RESULTS

HEMODYNAMIC RESPONSE TO EXERCISE

The heart rate response to exercise is shown in figure 3. In both control and transplant dogs, heart rate was significantly elevated ($p < 0.05$) from the onset of exercise to 15 minutes of recovery. No significant differences were observed between groups at any time. As well, there was no significant difference in the maximal exercise induced heart rates for control (241 ± 8.7) and transplant (246 ± 6.7).

The hematocrit response to exercise is shown in figures 4a and b. Hematocrit was significantly elevated over pre-exercise values at 15, 45, and 60 minutes in controls and 15-60 minutes in transplants. When expressed as absolute values there were no significant differences between groups; however, when the data was expressed as delta scores, the transplants exhibited significantly ($p < 0.05$) higher responses than controls at 30, 45, and 60 minutes. Hematocrit and epinephrine were significantly correlated in both controls ($r = 0.66$, $p < 0.001$) and transplants ($r = 0.56$, $p < 0.001$) (see Fig. 5).

ISLET HORMONE RESPONSE TO EXERCISE

The insulin response to exercise is displayed in figures 6a and b. No significant differences were observed between groups or from baseline in either group during exercise using either absolute or delta scores. As can be observed in figure 6b, the insulin response to exercise in the two groups was markedly dissimilar, if not statistically significant. Both controls and transplants exhibited an initial decrease, significant

($p < 0.05$) in transplants in insulin levels which was maintained in the controls, but only transient in transplants where the initial decrease was followed by an elevation at 30 and 45 min. In recovery, insulin was significantly ($p < 0.05$) lower than pre-exercise levels in both control and transplanted dogs.

Absolute and delta c-peptide responses to exercise are displayed in figures 7a and b. When expressed as absolute values, controls were consistently higher than transplants, although no significant differences were observed between groups. As well, no significant differences from baseline were observed for controls while transplants were significantly different from baseline at 30 min of recovery. As illustrated in figures 6b and 7b, c-peptide and insulin followed similar trends and significant ($p < 0.001$) correlations were observed in both control ($r = 0.87$) and transplanted ($r = 0.69$) dogs (see Fig. 8).

Absolute and delta glucagon responses to exercise are displayed in figures 9a and b. Controls exhibited significantly ($p < 0.05$) elevated glucagon levels at 30, 45, 60, 75, and 90 minutes, while transplants exhibited significantly elevated glucagon levels at 45, 60, and 75 minutes. Although mean delta glucagon levels in transplants were consistently twofold higher than controls, no significant differences were observed between groups. In transplants, glucagon was very strongly correlated with epinephrine ($r = 0.81$, $p < 0.001$) (see Fig. 10) and hematocrit ($r = 0.77$, $p < 0.001$) (see Fig. 11), while no significant correlations were observed in controls.

PLASMA GLUCOSE RESPONSE TO EXERCISE

Figures 12a and b show the effects of exercise on plasma glucose

concentration. Plasma glucose fell significantly ($p < 0.05$) from pre-exercise levels at the onset of exercise in both control and transplant dogs. Plasma glucose levels remained significantly depressed until 30 minutes post-exercise in controls and did not return to baseline in the transplants. As illustrated in figure 12a, the absolute plasma glucose levels were very similar in control and transplants during exercise and no significant differences were observed between the two groups at any time point. When the data was expressed as delta scores, as illustrated in figure 12b, transplanted dogs exhibited consistently lower glucose levels than controls and these differences were significant ($p < 0.05$) at 15 and 30 min of recovery.

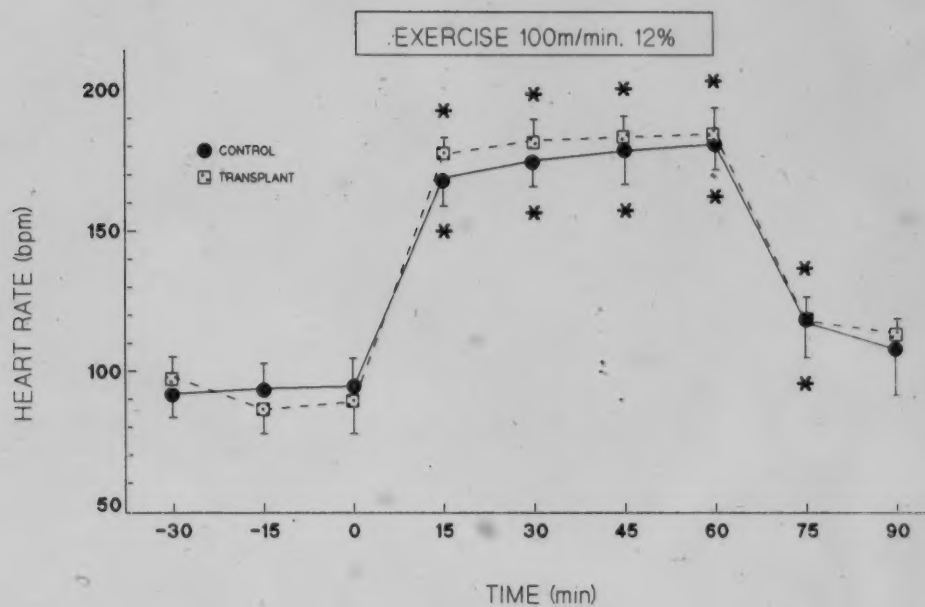
SYMPATHOADRENAL RESPONSE TO EXERCISE

Epinephrine responses to exercise are displayed in figures 13a and b. Epinephrine was significantly ($p < 0.05$) elevated from baseline in controls at 30 and 60 minutes and in transplants at 15, 30, and 60 minutes. Although no significant differences were observed between groups at any time point, mean delta epinephrine in transplanted dogs was fivefold greater than controls at 45 and 60 minutes. Norepinephrine responses to exercise are illustrated in figures 14a and b. Norepinephrine was significantly ($p < 0.05$) elevated in controls from 15 through 60 minutes and 15, 45, and 60 minutes in transplants. No significant differences were observed between groups at any time point with data expressed as absolute or delta scores.

PLASMA LACTATE RESPONSE TO EXERCISE

Lactate responses to exercise are shown in figures 15a and b.

Plasma lactate was significantly elevated from baseline in controls at 30 to 75 minutes and in transplants at 45 to 75 minutes. No significant differences were observed between groups at any time point, although mean values for transplants were consistently higher than controls.

**Fig. 3**

Heart rate response to exercise (mean $\pm$ SEM) * significant ($p < 0.05$) difference from baseline

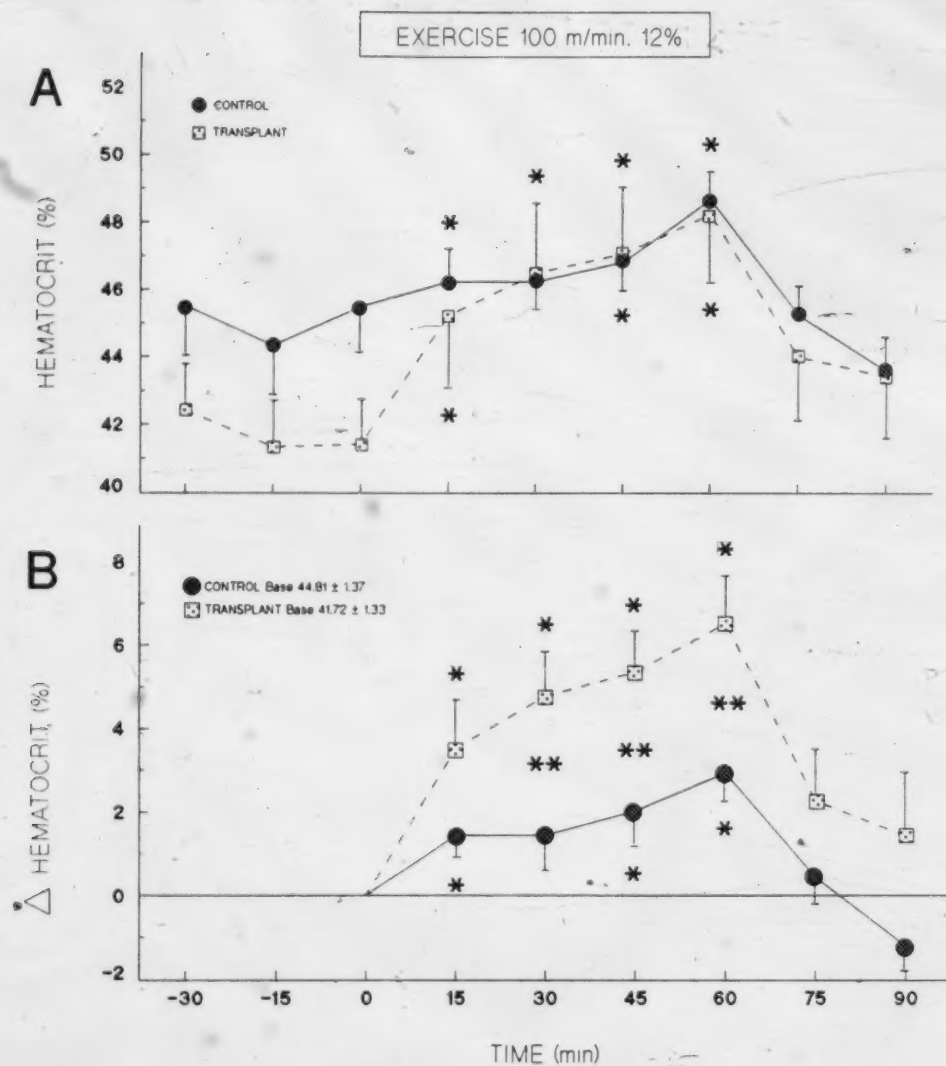


Fig. 4

Hematocrit response to exercise a) absolute means $\pm$ SEM b) mean delta scores $\pm$ SEM *significant ($p < 0.05$) difference from baseline **significant ($p < 0.05$) difference between groups

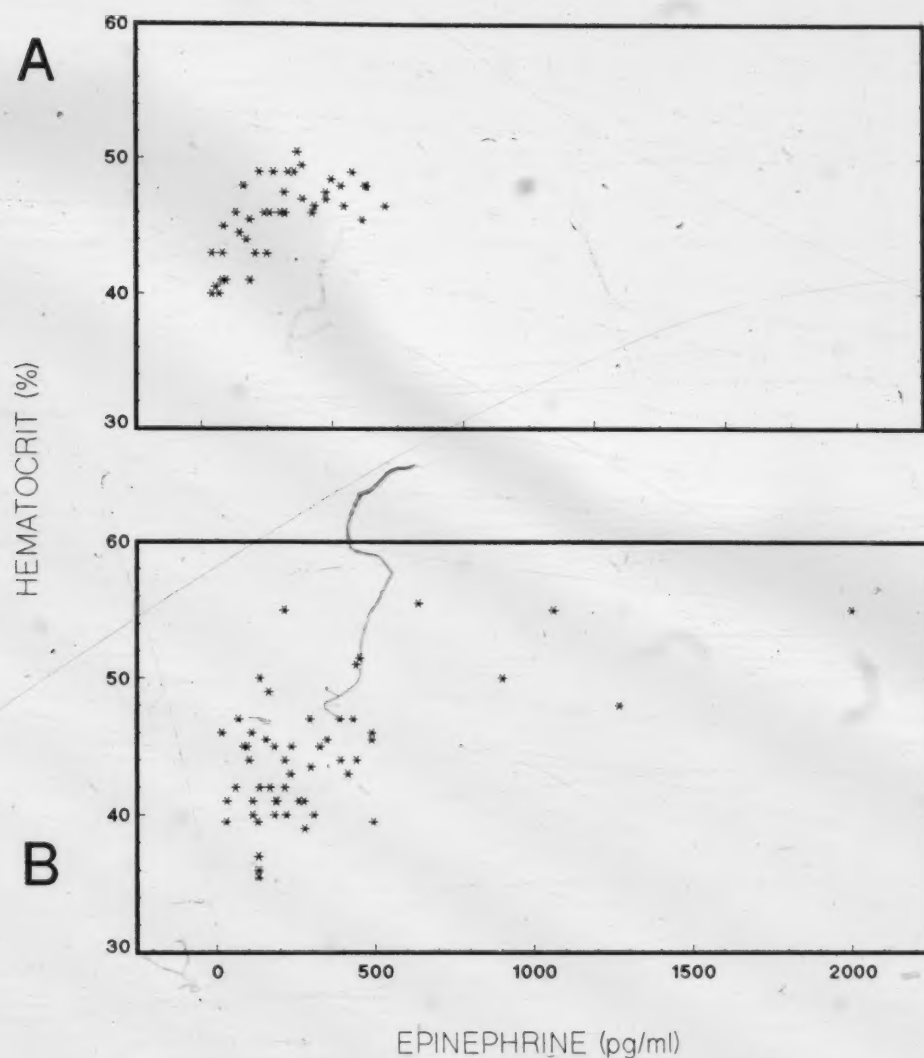
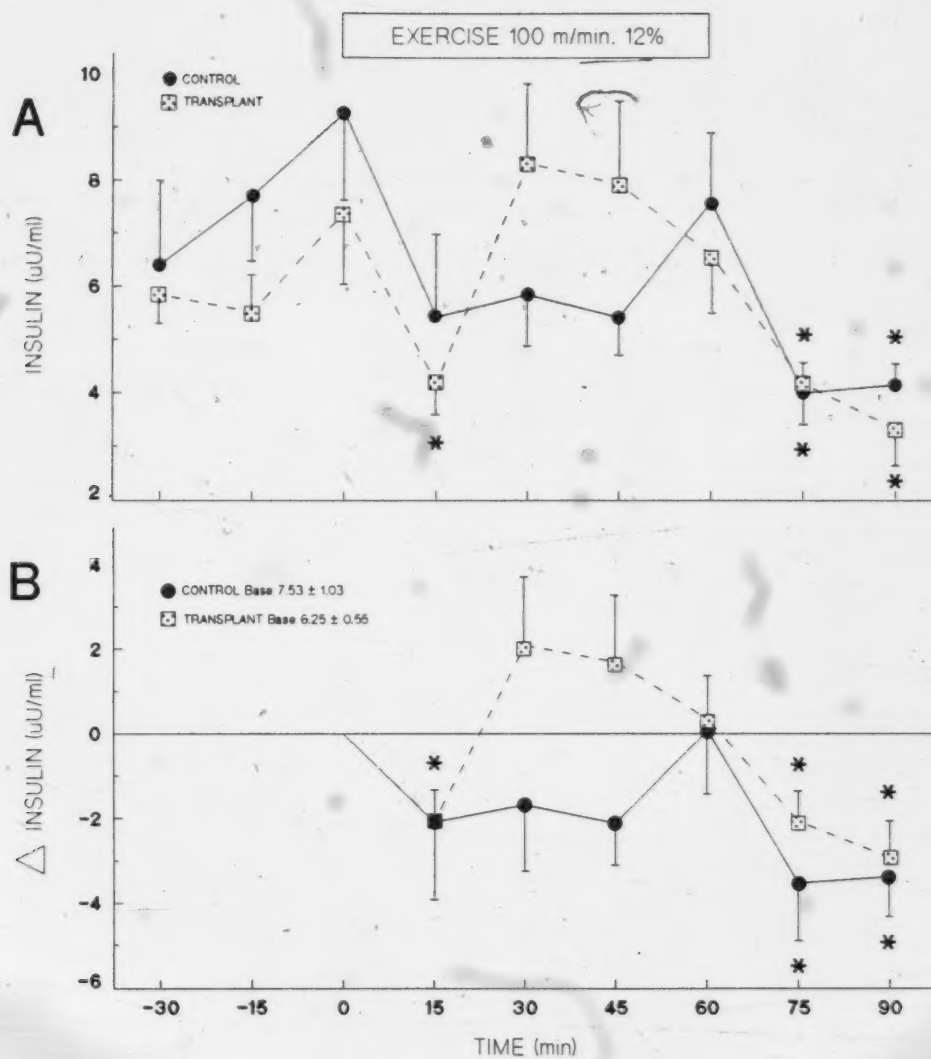


Fig. 5

Scattergram of epinephrine and hematocrit a) control dogs ($r=0.66$, $p<0.001$, $n=42$) b) transplant dogs ($r=0.56$, $p<0.001$, $n=53$) outliers in transplant group (epinephrine > 1000 pg/ml) occurred in three different dogs

**Fig. 6**

Insulin response to exercise a) absolute means $\pm$ SEM b) mean delta scores $\pm$ SEM *significant ($p < 0.05$) difference from baseline

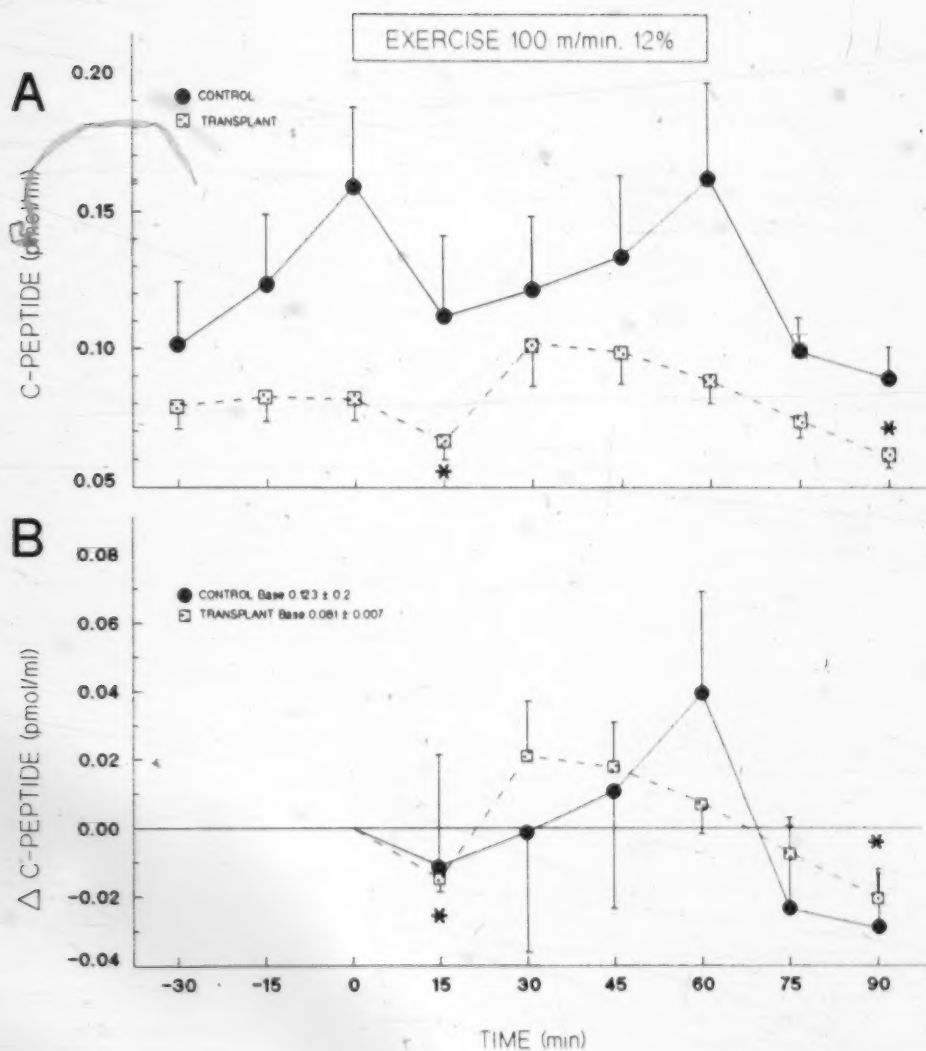
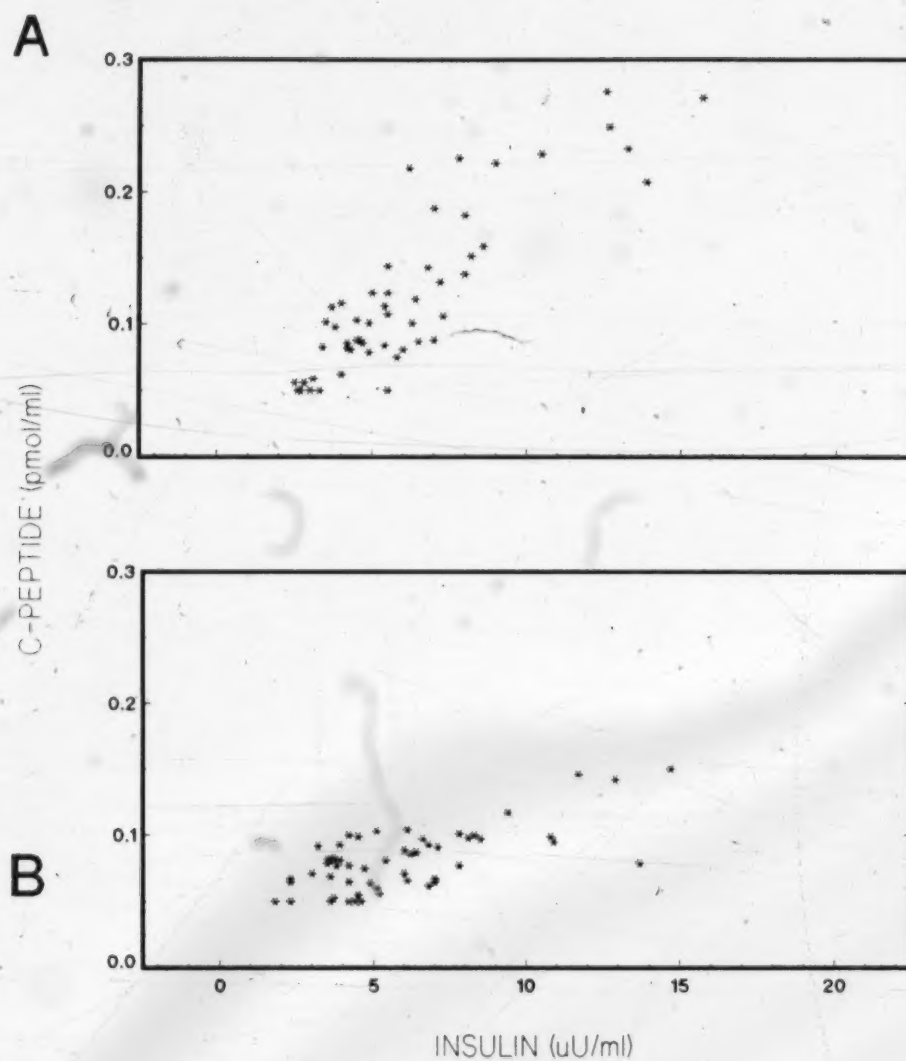


Fig. 7

C-peptide response to exercise a) absolute means $\pm$ SEM, b) mean delta scores $\pm$ SEM * significant ($p < 0.05$) differences from baseline

**Fig. 8**

Scattergram of c-peptide and insulin a) control dogs ($r=0.87$, $p<0.001$, $n=52$) b) transplanted dogs ($r=0.69$, $p<0.001$, $n=54$)

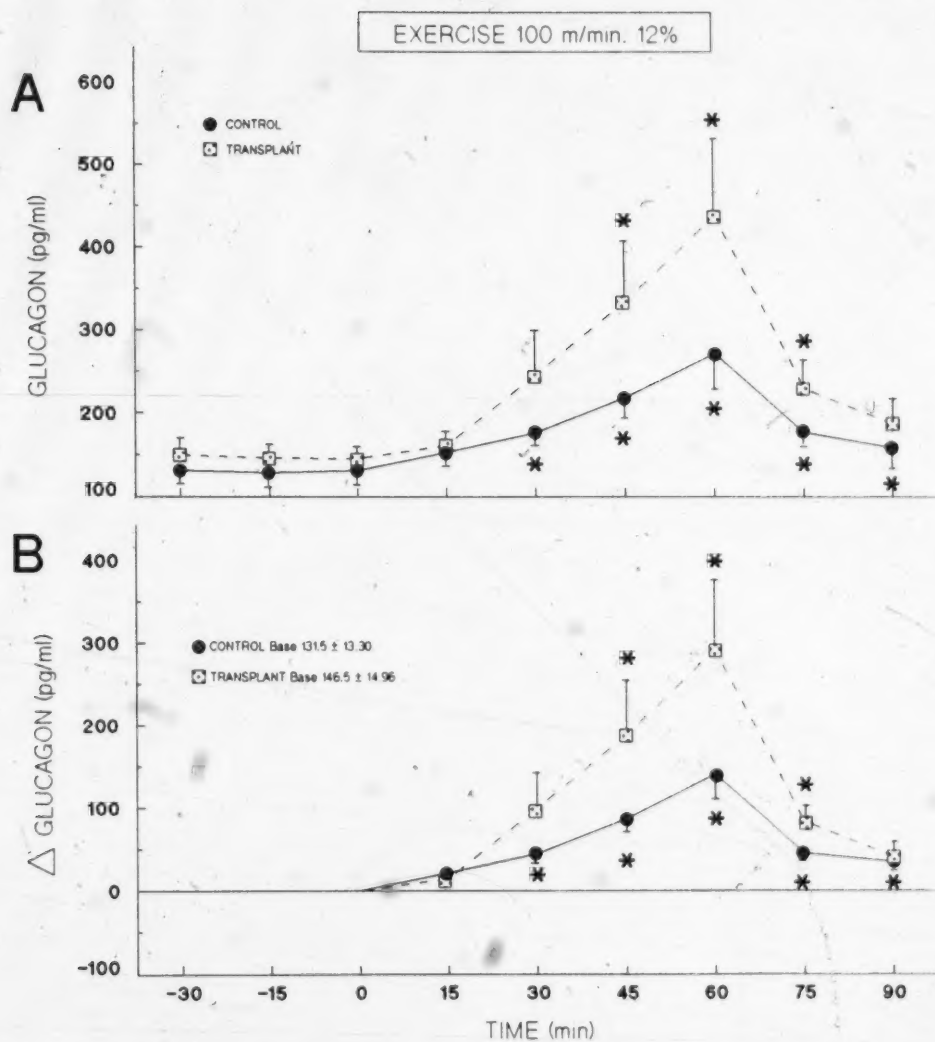


Fig. 9

Glucagon response to exercise a) absolute means $\pm$ SEM, b) mean delta scores $\pm$ SEM * significant ($p < 0.05$) difference from baseline

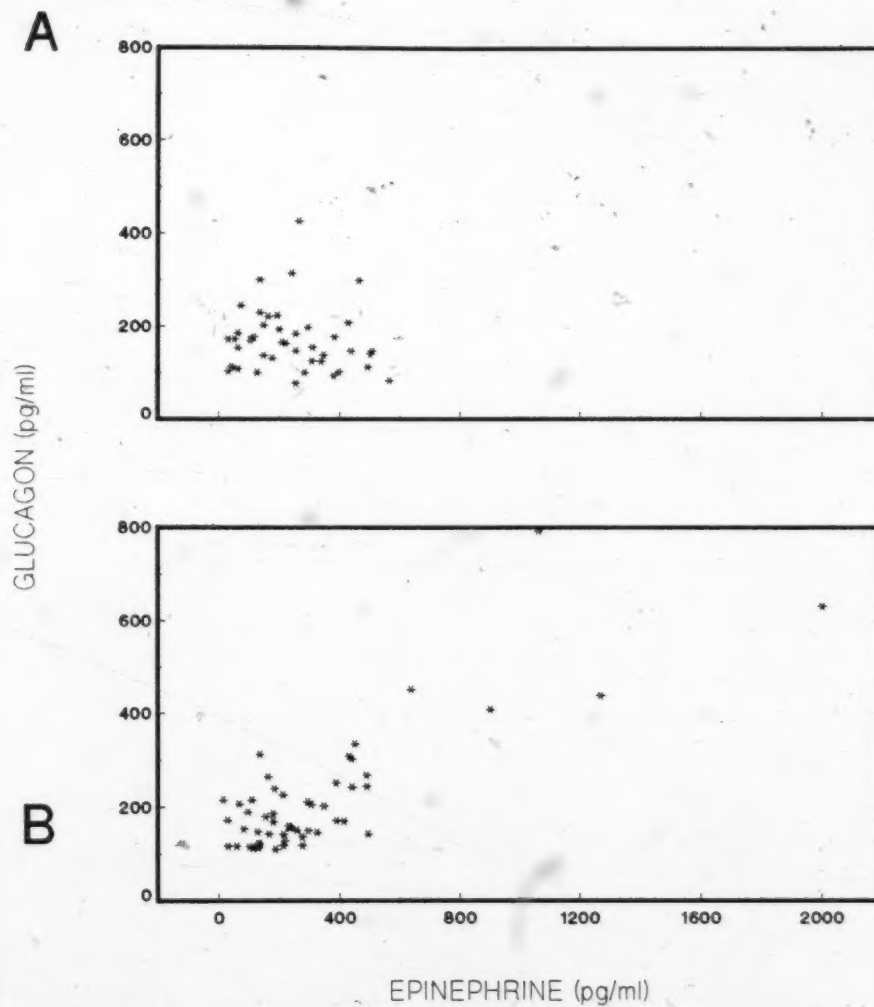


Fig. 10

Scattergram of glucagon and epinephrine a) control dogs ($r=-0.11$, $p=0.24$, $n=42$) b) transplanted dogs ($r=0.81$, $p<0.001$, $n=53$) outliers (epinephrine > 1000 pg/ml) occurred in three different transplanted dogs

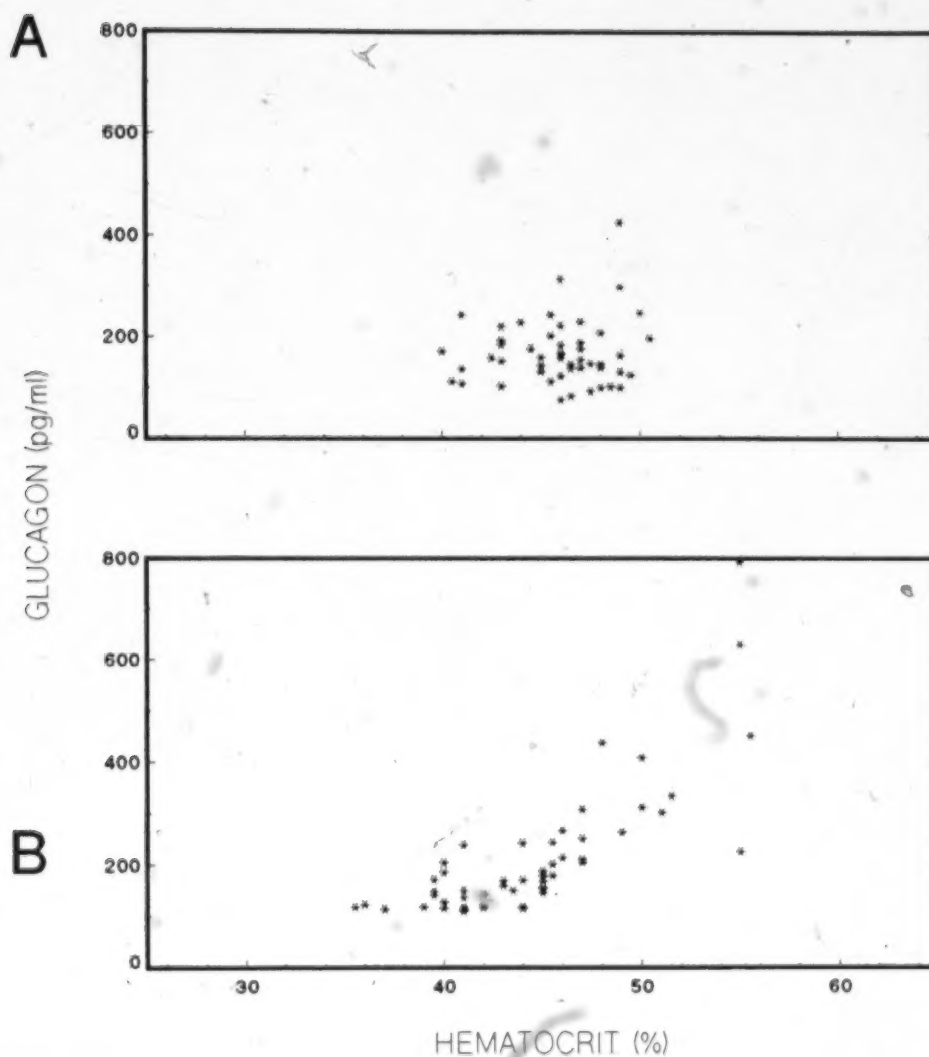


Fig. 11

Scattergram of hematocrit and glucagon a) control dogs ($r=0.10$, $p=0.235$, $n=51$) b) transplanted dogs ($r=0.77$, $p<0.001$, $n=54$)

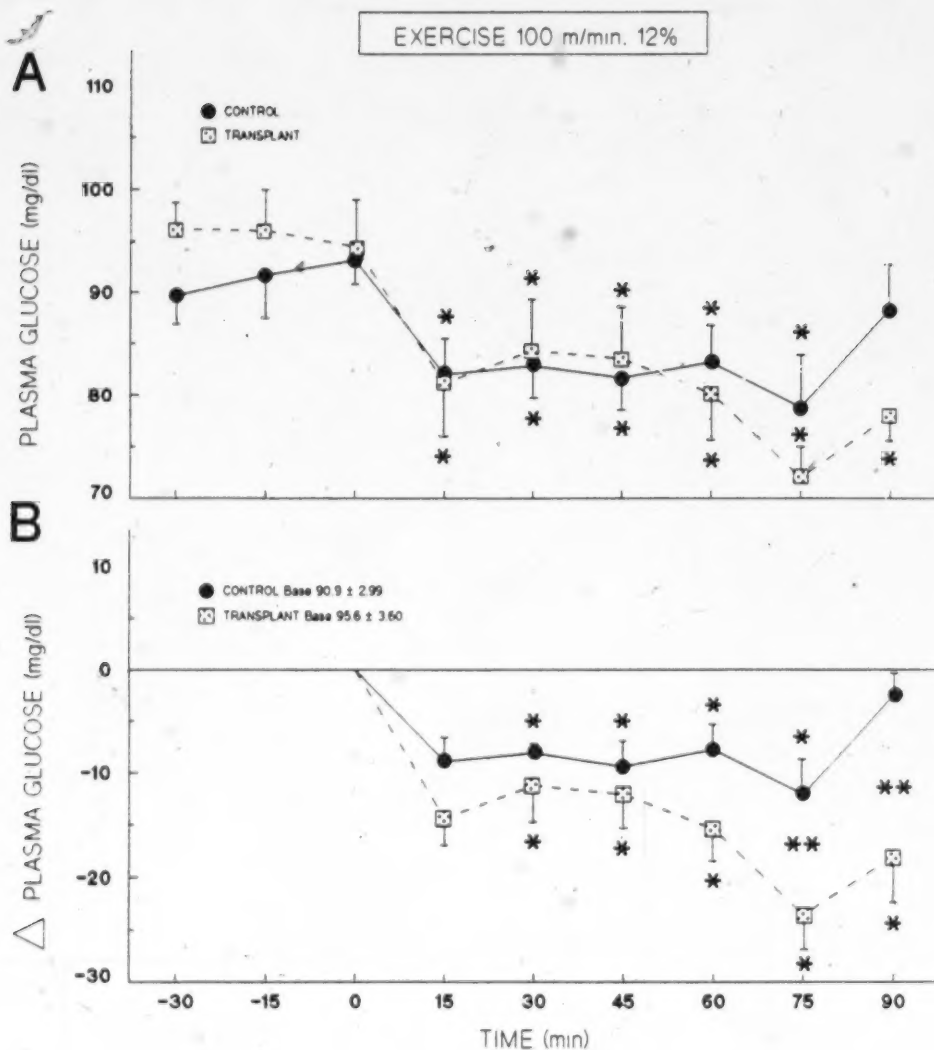


Fig. 12

Plasma glucose responses to exercise a) absolute means $\pm$ SEM, b) mean delta scores $\pm$ SEM * significant ($p < 0.05$) difference from baseline
** significant ($p < 0.05$) difference between groups

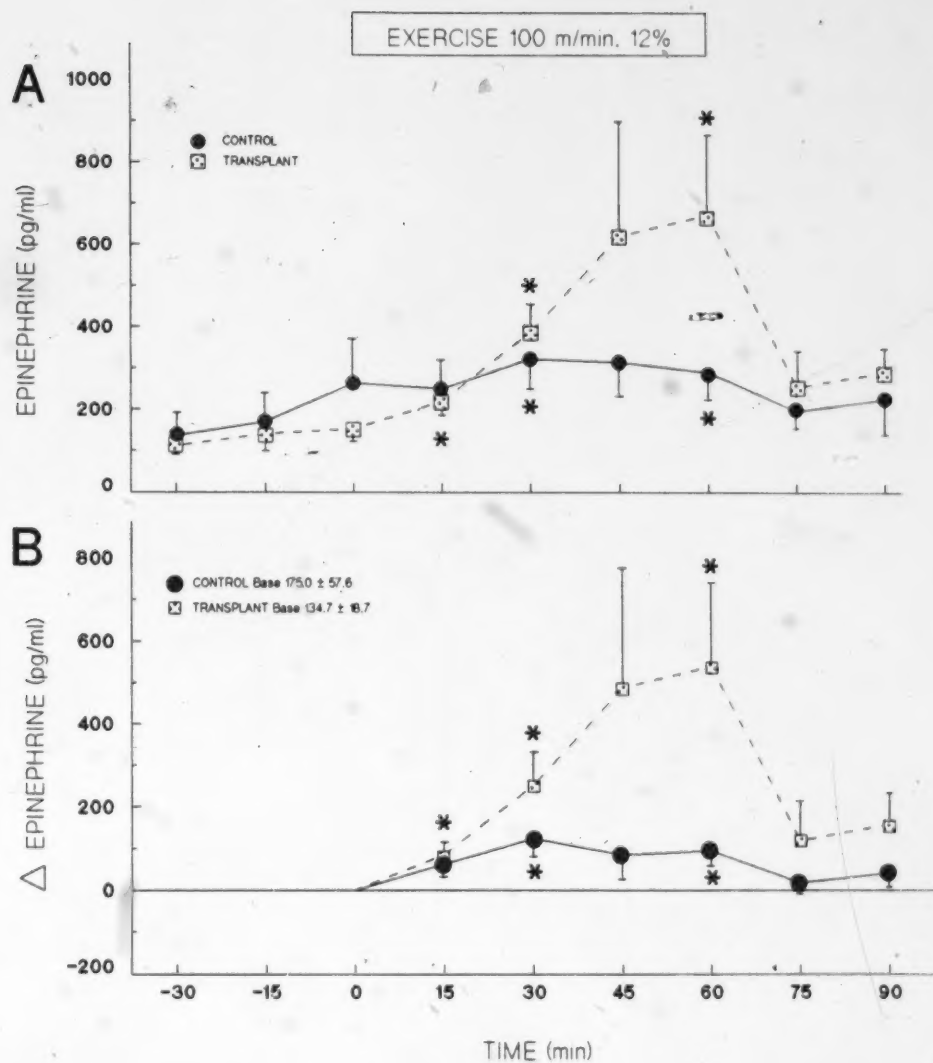


Fig. 13

Epinephrine responses to exercise a) absolute means $\pm$ SEM, b) mean delta scores $\pm$ SEM. * significant ($p < 0.05$) difference from baseline

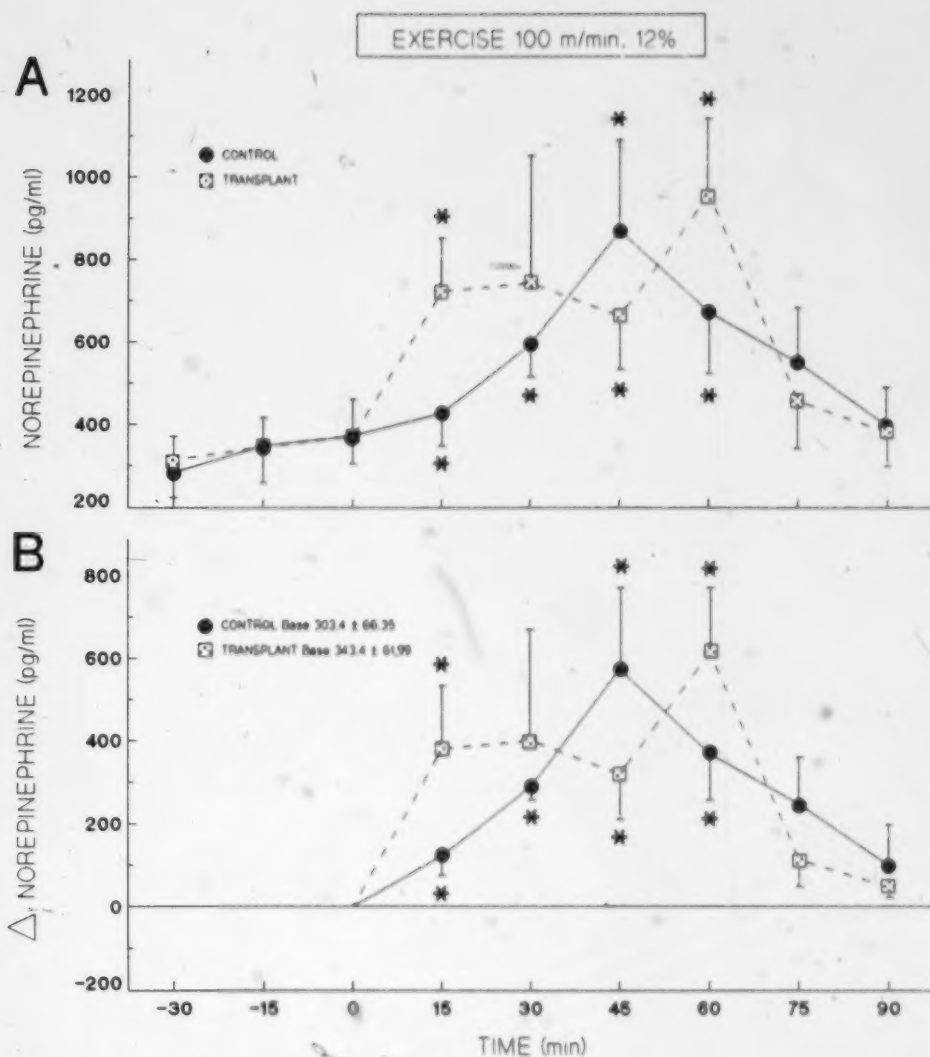


Fig. 14

Norepinephrine responses to exercise a) absolute means $\pm$ SEM, b) mean delta scores $\pm$ SEM * significant ($p < 0.05$) difference from baseline

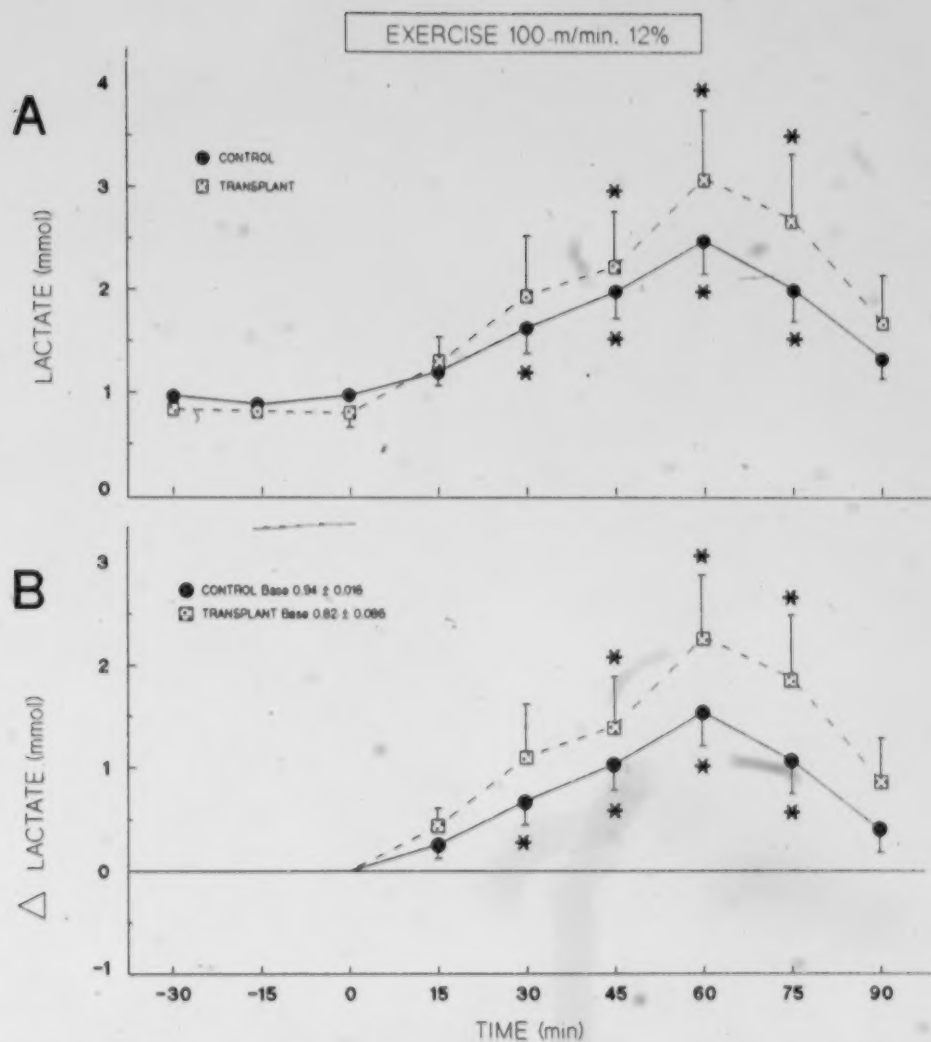


Fig. 15

Plasma lactate response to exercise a) absolute means $\pm$ SEM, b) mean delta scores $\pm$ SEM * significant ($p < 0.05$) difference from baseline

IV DISCUSSION

This study was designed to observe the impact of transplantation of isolated islets of Langerhans on the glucoregulatory hormone response to exercise in depancreatized dogs. It has been previously shown that the isolated and transplanted islet cells respond adequately, if not optimally, to changes in glucose concentration during oral and intravenous glucose tolerance tests (Rajotte et al 1984, Warnock et al 1987). However, the capacity of the transplanted islet cells to respond to neurohormonal control has not been previously addressed. Because of the well characterized pancreatic endocrine response which occurs in the absence of large changes in glucose levels (Cochran et al 1966, Bottger et al 1972, Vranic et al 1976, Wasserman et al 1984), exercise was selected as a model where neurohormonal responses could be examined without the complication of nutrient stimuli.

On most factors examined in this study, the transplanted dogs displayed greater variability than the controls. Largely because of this variability, statistical significance was rarely achieved between groups, although the means were apparently quite different. While hormonal measures are characteristically quite variable, as can be observed in insulin and c-peptide in the controls at rest, the variability in the counterregulatory responses in the transplanted dogs was unusually large and it is essential that this variability be accounted for. While the islet cell autografted dog has been a consistently reproducible, almost routine, model in this lab, there is still a considerable source of variability in the transplantation

procedure. The number of islets a dog receives is dependent upon the yield from the islet cell isolation, which is variable between dogs. This variation in islet cell mass is further compounded by a variable degree of revascularization and function of the grafted islets. Reinnervation of autografted islets has been reported well within the post-transplant period of the dogs in this study (Madureira et al 1985). It is conceivable that the autografts may have become reinnervated in the dogs observed in this study. However, there is no certainty that reinnervation, if it occurred, approximated the full physiological complement of cholinergic, adrenergic, and peptidergic innervation. As well, there is no certainty that all dogs underwent a similar extent or nature of reinnervation.

We are confident, however, that the variability between dogs was not a product of differences in exercise capacity. Heart rate responses to the imposed exercise intensity were consistent between control and transplants and are consistent with those previously observed for exercise of this intensity (Ordway et al 1984). As well, the maximal heart rates observed from the progressive intensity test were also consistent between groups and with the literature. The equality of the relative intensity of the imposed exercise is further supported by the norepinephrine levels, which can be interpreted as an indicator of hemodynamic stress during exercise (Christensen and Galbo 1983), which were similar between groups and consistent with the literature (Wasserman et al 1984).

While not significantly different, due to the large variability, the insulin and glucagon responses to exercise were qualitatively different between transplants and controls. The mean glucagon response

in the transplanted dogs, was consistently twofold greater than that observed in controls. The mean insulin response was initially similar in control and transplanted dogs but midway through the exercise bout the transplants exhibited an increase in insulin concentration while the transplants remained suppressed. These responses are undoubtedly qualitatively different, but the problems of why these differences occurred and whether they had an impact on the glucoregulatory status of the transplanted dogs arise.

ISLET CELL RESPONSE TO EXERCISE

The elevated glucagon response to exercise in the transplanted dogs can largely be attributed to the effects of epinephrine. A very strong correlation was observed between epinephrine and glucagon levels in the transplants ($r=0.81$, $p<0.001$) which was not observed in the controls. The glucagon response to exercise has previously been prevented by β adrenergic blockade (Lefebvre and Luyckx 1974, Galbo et al 1976, 1977, Simonson et al 1984) but was not affected by adrenalectomy (Jarholt and Holst 1979, Hoelzer et al 1986b), suggesting that the response can be mediated through sympathetic neural mechanisms. A correlation between epinephrine and glucagon was not observed in the controls, and has not been cited in the literature, which suggests that the normal pancreas does not merely respond to the increased adrenergic drive associated with exercise, but is under specific central coordination, distinct from that regulating epinephrine, in response to an integration of the glucoregulatory status of the exercising animal. If this is indeed the case, then the autografted islets cannot be considered to be responding under direct hypothalamic control but, instead, were merely driven by

circulating epinephrine levels. While these results do not support the case of physiologic glucoregulation by autografted islets, they do provide evidence that the transplanted A cells are functional because, while normal basal glucagon levels have been observed in depancreatized dogs (Doi et al 1976) from extrapancreatic sources, it has been observed that extrapancreatic glucagon did not respond to exercise (Vranic et al 1976).

The unexpected insulin response to exercise in the transplanted dogs is difficult to explain. The control response to exercise was basically consistent with previous studies (Vranic et al 1976, Wasserman et al 1984), but the basal insulin levels in both control and transplants were lower than those previously observed. This may be due to differences in diet in other labs, or to sampling differences between arterial and venous blood. We are confident in our results as they were verified externally (courtesy of R. Gingerich, Washington University, St. Louis) and were strongly correlated with c-peptide levels in both control ($r=0.87$, $p<0.001$) and transplant ($r=0.69$, $p<0.001$) groups. If expressed as percentage change, the control group results are consistent with the literature, but perhaps because of the low initial values these changes were not significant.

Insulin secretion has not previously been observed to increase during exercise in dogs. Because the initial insulin response was similar in both control and transplant dogs and the groups differed subsequently, it is tempting to suggest that there may be a two phase control of insulin secretion where early in exercise insulin levels are suppressed by a given mechanism, but prolonged suppression is mediated by a different mechanism. The initial phase of insulin suppression may

be mediated through adrenergic mechanisms which can be interchangeably driven by circulating or neural sources. The continued suppression of insulin secretion may be mediated by alternate mechanisms, possibly neuropeptidergic, of which there are several potential effectors, such as galanin (Dunning et al 1986, McDonald et al 1985, 1986), pancreastatin (Tatemoto et al 1986), calcitonin gene-related polypeptide (Pettersson et al 1986) and neuropeptide Y (Pettersson et al 1987). The results observed in this study could be explained by such a biphasic mechanism of insulin suppression. Despite denervation and regardless of potential reinnervation, the transplanted islets are still subject to adrenergic stimulation via circulating epinephrine. The inability of the transplanted dogs to continue suppression of insulin levels may be secondary to the loss of peptidergic innervation. Present knowledge regarding the normal physiology of the peptidergic role in islet regulation is insufficient to discuss the potential for peptidergic reinnervation of the autografted islets or peptidergic stimulation through non-synaptic sources.

It is unlikely that β adrenergic stimulation (Samols and Weir 1979, Miller 1981) of the B cells was responsible for the increase in insulin levels during exercise in the transplanted dogs because of the recent observations in purified B cells which indicated that the B cells were unresponsive to β adrenergic stimulation (Schuitt and Pipeleers 1986). In light of these recent observations it appears that the previously observed β adrenergic stimulation of B cell secretion may have been an artifact of β adrenergic stimulation of the A cells, which resulted in an increase in glucagon levels which then stimulated insulin secretion. Similarly, in this study, it is possible that the increase in insulin

levels observed in the transplanted dogs during exercise may have been an indirect response to β adrenergic stimulation of the autografted A cells. However, in the transplanted dogs, insulin and glucagon were weakly correlated ($r=0.31$, $p=0.013$) and c-peptide and glucagon were not significantly correlated ($p>0.05$).

It is also possible that the elevated insulin levels occurred secondarily to splenic contraction. The normal exercise response in dogs includes an α adrenergically mediated splenic contraction, which is thought to play a role in increasing the oxygen carrying capacity of the blood through an increase in hematocrit (Longhurst et al 1986). Because the autograft was placed in the spleen, it is possible that B cell secretion did not actually increase, but that insulin was "wrung out" of the spleen with the red cells. If this were the case, insulin and glucagon levels should show a similar response to the nonspecific effects of splenic contraction because they are originating from the same islets. However, insulin was weakly correlated with hematocrit ($r=0.35$, $p=0.005$) and no correlation was observed for c-peptide ($p>0.05$). It is interesting that in the transplanted dogs glucagon levels were similarly correlated with both epinephrine ($r=0.81$, $p<0.001$) and hematocrit ($r=0.77$, $p<0.001$).

It is possible that insulin and glucagon were not squeezed out of the spleen upon exercise induced contraction, but that the autografted A and B cells responded to changes in regional blood flow or secondarily to metabolic changes in the adjacent splenic tissue coincident with contracture. If the unusual insulin and glucagon responses observed in the transplanted dogs are the result of local environmental factors, as opposed to general splenic contraction, there is no reason to believe

that these factors might not have different effects on A and B cells. As well, these effects might result from regional contracture, which might not be detected in hematocrit changes and might not affect all islets in all dogs. Unfortunately, the mechanism underlying the differential response of insulin and glucagon secretion in transplanted dogs can only be speculated from this data.

GLUCOREGULATORY RESPONSE TO EXERCISE

It is difficult to state, from the present data, whether the unusual responses in insulin and glucagon had an effect on glucoregulatory status during exercise in the transplanted dogs. Absolute glucose levels fell significantly ($p < 0.05$) in both control and transplanted dogs. A general decrease in plasma glucose is consistent with previous observations (Vranic et al 1976, Wasserman et al 1984), although the fall was not statistically significant in these studies. The mean data suggests the following scenario, which is consistent with current literature. In this schema, the elevated insulin response inhibits hepatic glucose production (Kawamori et al 1977, Felig and Wahren 1979), which results in hypoglycemic stress which results in an elevated epinephrine response (Wasserman et al 1984, Wolfe et al 1986) which then stimulates muscle glycogenolysis and lactate production (Issekutz 1984). As well, the elevated epinephrine levels stimulate greater glucagon release (Miller 1981, Schuit and Pipeleers 1986) which in turn further stimulate insulin release and the spiral continues. While the mean data appears to strongly support this interpretation, the raw data does not indicate that the unusual insulin response is the primary defect.

While glucose kinetics were not examined in this study, some indications of the glucoregulatory status of the transplanted dogs may be surmised from other variables. The mean epinephrine response was markedly higher in the transplants than in controls, although this difference was not significant, presumably because of the large variability in the transplanted dogs. Epinephrine is considered to respond to hypoglycemic stress (Christensen and Galbo 1983, Ungar and Phillips 1983) and has been previously observed to be greatly elevated in dogs (Wasserman et al 1984) and humans (Wolfe et al 1986) during hypoglycemic exercise. The high epinephrine levels observed in the transplanted dogs suggest that, as a group, they experienced a degree of glucoregulatory distress during exercise. Epinephrine has previously been observed to increase hepatic glucose production and inhibit glucose utilization in exercising dogs (Wasserman et al 1984, Issekutz 1985). It would then follow that the transplanted dogs experienced difficulty in matching glucose production to glucose utilization and, in an attempt to maintain relative normoglycemia, relied upon an increase in epinephrine to decrease muscle glucose utilization and increase hepatic glucose production so that the two processes might be matched. However, in the transplanted dogs, decreases in glucose were not strongly correlated with increases in epinephrine as one might expect if hypoglycemia were the stimulant for the elevated epinephrine. Regarding this point, a degree of caution must be observed as epinephrine peaks, with a half life of approximately 30 seconds in circulation, may have occurred within the 15 minute sampling interval and been missed.

The epinephrine induced decrease in muscle glucose utilization during exercise is believed to be achieved in part by a stimulation of

muscle glycogenolysis (Issekutz et al 1978, 1984). The increase in muscle glycogenolysis is believed to decrease muscle glucose utilization, not only by providing an alternate metabolic substrate to plasma glucose, but also by increasing the intracellular pool of glucose-6-phosphate, which is believed to strongly inhibit hexokinase and consequently decrease muscle uptake of blood glucose (Issekutz 1980). It has previously been observed that epinephrine causes an increase in lactate production from muscle glycogen during exercise in dogs (Issekutz 1984). This is in agreement with the consistently higher plasma lactate levels observed in the transplanted dogs. Plasma lactate and epinephrine levels were correlated to a similar extent in control and transplanted dogs. Although not conclusive in isolation, the elevated mean plasma lactate in the transplanted dogs, relative to controls, provides additional support for the interpretation from the epinephrine observations that the transplanted dogs, as a group, experienced a degree of hypoglycemic distress during exercise. However, also in keeping with the epinephrine observations, lactate levels in the transplanted dogs were weakly correlated with plasma glucose concentrations ($p > 0.05$). Caution in interpreting this statistical observation is warranted because there is a delay between adrenergic stimulation, metabolic production of lactate and its appearance in the blood.

It is extremely difficult from the present data to conclude whether the the unusual responses in insulin and glucagon had an impact on the glucoregulatory status of the transplanted dogs. It is doubtful that elevated insulin secretion in the transplants caused an inhibition of hepatic glucose production triggering a compensatory epinephrine

response, because there was no correlation between insulin and epinephrine levels. Similarly, it is possible that the elevated glucagon levels might also have been without effect. Presently there is considerable confusion regarding the importance of the pancreatic response to exercise. Supporting a central role for insulin and glucagon in the glucoregulatory response to exercise are the observations that hyperinsulinemia, through inhibition of hepatic glucose production (Kawamori and Vranic 1977, Felig and Wahren 1979, Wolfe et al 1986), and hypoinsulinemia, through overstimulated hepatic glucose production (Issekutz 1980), both have an impact on glucose kinetics and result in departures from normoglycemia. Similarly, hypoglucagonemia during exercise has been observed to markedly decrease hepatic glucose production and result in hypoglycemia (Issekutz and Vranic 1980, Wasserman et al 1984). However, the recent studies from Cryer and co-workers in humans, where the glucoregulatory response to exercise was examined during maintenance of fixed insulin and glucagon levels and/or combined α and β adrenergic blockade, strongly suggested that the pancreatic and sympathoadrenal responses were redundant (Hoelzer et al 1986a, b, Tuttle et al 1987). It is possible that there may be species specific differences in the nature of the glucoregulatory response to exercise which have not yet been elucidated.

While no firm conclusions can be stated without glucose kinetics data, it appears, from the raw data, that within the transplanted group there was a range of glucoregulatory sensitivity, whereby some dogs were sensitive to the relatively mild hypoglycemia and responded appropriately, while other dogs were apparently insensitive to their glucoregulatory distress. Although the islet cell autografted dogs are

consistently normoglycemic at rest, they also consistently exhibit poorer glucose tolerance than normal dogs (Warnock et al 1983, Rajotte et al 1984, Warnock et al 1987). The diminished glucose tolerance has been attributed to a number of factors including decreased islet cell mass, the ectopic location of the islets, and to islet denervation (Sutherland et al 1984). The insufficient glucose control in the transplanted dogs may underlie the delayed return to normoglycemia observed after exercise. Such a deterioration of glucoregulatory control would not be inconsistent with a prolonged period of diminished, though adequate, control of blood glucose levels.

The primary defect in the transplanted dogs which underlies the unusual metabolic response to exercise appears to be related to the diminished glucose tolerance, which may be a symptom of a larger problem. Homeostatic control of any physiological process requires the following three basic features. There must be an efferent mechanism by which control can be exerted over the process in question. There must also be an afferent mechanism so that the efferent activity may be graded in relation to requirement. Finally, there must be an integrative centre where the afferent and efferent mechanisms are coordinated. It is possible that changes in glucoregulatory capacity of the transplanted islets, relative to an intact pancreas, result in a loss in efferent potency, which may in turn limit glucose tolerance in autografted dogs. Over an extended period, the decrement in glucose tolerance may effect the afferent pathways (central and peripheral glucoreceptors) which may in turn effect the integrative center (ventromedial and ventrolateral regions of the hypothalamus) so that the homeostatic capabilities of the system are muted. Variance between dogs

may be related to the degree or nature of central glucoregulatory changes. It is quite possible that two or more sub-populations may be represented within the group of autografted dogs examined in this study which could not be identified because of the size of the present sample.

Although the preliminary nature of this study precludes strong conclusions, the major contribution to the present body of knowledge is as a guide for further research. It appears that the isolated and autografted islets of Langerhans are no longer under direct central control. Whether this had a direct impact on glucose kinetics during exercise in the transplanted dogs is uncertain from this study. While the transplanted dogs maintained plasma glucose at similar absolute levels to control dogs, they displayed unusual and variable counterregulatory responses which did not correlate with observable hypoglycemic distress suggesting that they may have undergone a fundamental glucoregulatory change post-transplant.

It is important to emphasize that these observations should not be interpreted to suggest that islet cell transplantation should be discarded as a therapy. As with any medical treatment there are side effects which must be examined in light of the benefit of the treatment. The potential of islet cell transplantation for improving and lengthening the lives of insulin dependent diabetics is so great that active research should continue. The value of studies such as this, lies in the attainment of greater knowledge about the physiological implications of islet cell transplantation so that potential recipients may make a more informed decision. As well with greater understanding of the side effects associated with islet cell transplantation steps may be taken to avoid or minimize them.

V SUMMARY AND RECOMMENDATIONS

This study was designed to examine two closely related but distinct questions. Firstly, whether autografted islets of Langerhans could respond to neurohormonal stimuli. Secondly, whether depancreatized dogs who were rendered normoglycemic by islet cell transplantation could maintain normoglycemia during exercise. In this study, physiologic, if not statistical, differences were observed between autografted and control dogs on both counts. These differences raise important questions regarding the potential for islet cell transplantation to truly "normalize" diabetic recipients.

ISLET HORMONE RESPONSES

The mean glucagon response to exercise in the transplanted dogs was markedly greater than that observed in controls. The elevated glucagon response in the transplanted dogs may have been the result of a functional deficit related to islet cell isolation or to environmental factors related to transplantation. More specifically, the strong statistical correlation observed between glucagon and epinephrine in the transplanted dogs, suggests that central control of glucagon secretion was disrupted. This disruption may have been the result of denervation inherent to islet cell isolation, or it may have been due to a decrement in central glucoregulatory capacity secondary to prolonged sluggish control of plasma glucose levels. Alternatively, the elevated glucagon response may have been the result of transplanting the islet cells to the spleen. Splenic contraction, characteristic of the normal canine hemodynamic response to exercise, may have "wrung" glucagon out of the

spleen with the red blood cells. It is also possible that environmental changes within the spleen, secondary to contracture, may have had a stimulatory effect upon the transplanted A cells.

The mean insulin response to exercise was initially similar in control and transplant dogs but, while the transplanted dogs exhibited a sustained suppression of insulin levels, the transplanted dogs exhibited an increase in insulin secretion over basal levels. This increase in insulin secretion, which has not been previously observed, differs qualitatively from the elevated glucagon response in the transplanted dogs. While the mean glucagon response was merely excessive in magnitude, the mean insulin response was opposite in direction, relative to control dogs.

There are several explanations for the unusual insulin response in the transplanted dogs. As with the glucagon response, they may have been the product of islet isolation or of environmental factors related to transplantation. The denervation inherent to islet isolation may have disrupted direct central control of insulin secretion. It is possible that there is a role for both adrenergic and peptidergic mechanisms in the exercise induced inhibition of B cell secretion. While the autografted B cells continue to be subject to adrenergic stimuli through circulating epinephrine, despite the loss of neurally released norepinephrine with denervation, they are probably no longer subject to neuropeptidergic regulation. This deficit of peptidergic stimuli may explain the mid-exercise surge in insulin secretion. Alternatively, the increased insulin secretion in the transplanted dogs may have been due to placement of the isolated islets in the spleen. Similarly to glucagon, insulin may have been "wrung" out of the spleen

upon contraction or the B cells may have been effected by environmental changes within the spleen, secondary to contracture. As well, the insulin response may have been the result of stimulation of the B cells by the elevated glucagon levels.

There are several experimental approaches which could be used to further investigate the observed phenomena. The effects of denervation on the islet hormone response to exercise could be further explored in a denervated pancreas model. As a surgical procedure, selective denervation of the pancreas would not differ greatly from the methods currently used to harvest a pancreas for islet cell isolation. Because the pancreatic innervation arrives with the vasculature, denervation could be accomplished by cutting the minor vessels and removing the nerve containing adventitia from the major vessels by a combination of dissection and ethanol painting (Kline et al 1980). Denervation could be confirmed by the absence of cephalic phases in insulin and pancreatic polypeptide responses to oral glucose.

To examine the role of splenic contraction in the present observations, an alternate transplantation site could be used. Potential sites include the liver and kidney capsule. While the liver has not previously been a successful site because of portal hypertension subsequent to transplantation (Miller et al 1983), there is optimism that recent advances in islet cell isolation, which have resulted in a greater purity of yield, may make it possible to avoid this problem. The kidney could also be used as a transplantation site, but because insulin would be secreted into peripheral, as opposed to the physiologic portal circulation, glucoregulatory implications might complicate observations.

GLUCOREGULATORY RESPONSES TO EXERCISE

The autografted dogs displayed physiologic, if not statistical, differences in plasma glucose, epinephrine and lactate responses relative to controls. Without glucose kinetics data the primary defect cannot be conclusively identified from these studies. However, the present data suggest that a diffuse glucoregulatory sluggishness, characteristic of the transplanted dogs, which resulted in a range of glucoregulatory sensitivity may have been a central factor.

To properly examine this issue several experimental approaches could be pursued. The present study should be replicated with measures of hepatic glucose production and metabolic clearance of glucose. Aside from the obvious requirement for these measures, there would be a second benefit by increasing the sample size whereby the variance in the transplanted group might be clarified. Sub-populations within the group, if they exist, might be identified with a greater sample size.

A second approach to studying the apparent glucoregulatory problems in the transplanted dogs is to dispense with the exercise model and design experiments to specifically address this issue. One of many appropriate approaches would be to study recovery from hypoglycemia in resting dogs, a counterregulatory response which involves both pancreatic and sympathoadrenal mechanisms. Cryer and co-workers (Clarke et al 1979, Gerich et al 1979) have previously addressed counterregulatory responses to insulin-induced hypoglycemia in humans. The basic procedures involved would not be unlike those presently used in this lab for intravenous glucose tolerance tests, with the only difference being infusing insulin (0.05 U/kg) instead of glucose. Optimally, glucose kinetics should be measured during this experiment

but it is not imperative. A good approach might be to commence a regular program where the transplanted dogs are subjected to hypoglycemic recovery test on a similar interval to oral glucose tolerance testing. If this were done, a post-operative profile of the glucoregulatory capacity of the transplanted dogs could be followed. To supplement this, a full examination of the glucose kinetic response to hypoglycemia could be performed on a smaller number of dogs.

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APPENDIX 1

REVIEW OF LITERATURE

A. ACUTE HORMONAL GLUCOREGULATORS

The regulation of blood glucose concentration within narrow limits receives a very high priority. At rest brain glucose consumption accounts for approximately one half of the postabsorptive hepatic glucose production (Huang et al 1980). Because neural function is impaired when blood glucose falls below 40 mg/dl (Sokal et al 1964), it is essential that hypoglycemia be prevented. If blood glucose levels are chronically elevated, it may lead to the complications associated with poorly controlled diabetes (Rossini 1976).

Blood glucose concentration is controlled at several levels but is considered to be coordinated by the ventromedial and ventrolateral regions of the hypothalamus (Frohman 1983, Oomura and Yoshimatsu 1984, Woods et al 1986). The control of blood glucose concentration can be considered to be a balance between glucose utilization and glucose appearance or production. Because of the intermittent nature of feeding, it is essential that there be a steady source of glucose to replenish blood glucose as it is depleted. The liver is responsible for glucose production through both glycogenolysis or gluconeogenesis. To maintain normoglycemia, it is essential that the processes of glucose production be matched to glucose utilization. In practice, the challenge to the glucoregulatory system is to match glucose production, which is quite variable, to glucose utilization, which is in essence a

product of metabolic demand and is determined by the activity of the periphery.

There are three major regulators, insulin, glucagon, and the sympathoadrenal system, which play a role in acute control of blood glucose metabolism. Insulin, which is released by the B cells of the pancreas, increases glucose utilization and decreases glucose production. Glucagon, which is released by the A-cells of the pancreas, exerts its effects primarily on the liver by stimulating glucose production. The sympathoadrenal system (SAS), comprised of the sympathetic nervous system (SNS) and the adrenal medulla, is capable of increasing glucose production and decreasing glucose utilization. During post-absorptive resting conditions, insulin and glucagon are the primary glucoregulators and are coordinated through feedback control from blood glucose concentration while the SAS plays an undefined role in supplementing glucagon. Other hormones which have effects on glucose metabolism, such as cortisol, growth hormone, angiotensin etc., are not considered in this review because their effects are exerted over an extended period and are not as potent (Woods et al 1986).

Insulin

Insulin is a polypeptide hormone produced by the B cells of the islets of Langerhans of the pancreas. The mature insulin molecule consists of two polypeptide chains, referred to as the A and B chains, which are attached by a pair of disulfide bridges. These two chains arise from a single polypeptide through the post-translational removal

of a peptide from the centre. This removed peptide is secreted in equimolar amounts with mature insulin and is referred to as connecting or c-peptide. Because of this equimolar release, c-peptide has been used as an indicator of insulin secretion (Polonsky et al 1986). Insulin has a half life in circulation of approximately 5 minutes. In the dog, both the liver and kidney extract approximately 50% of the insulin which passes through these tissues and individually they clear approximately 52% and 27% of the total insulin secreted by the B cells (Polonsky et al 1983a). Because insulin clearance by both the liver and kidney was observed to be saturable at high insulin concentrations, it has been suggested that a receptor dependent mechanism is involved. C-peptide in the dog is cleared almost entirely by the kidney and has a half life of approximately 5 minutes (Polonsky et al 1983b). Human c-peptide has a considerably longer clearance time of approximately 30 minutes (Faber et al 1979), which has been attributed to the fact that human c-peptide is considerably larger, 33 residues, than dog c-peptide, 23 residues (Polonsky et al 1986).

Insulin has been referred to as a hormone of abundance because its secretion rises when there is an excess of circulating metabolic substrates. Insulin's actions are directed towards reducing the level of these substrates back to post-absorptive levels and facilitating their storage. More specifically, insulin stimulates the cellular uptake of glucose, free fatty acids and amino acids from the blood. Once these substrates are taken up, insulin facilitates their storage by enhancing the synthesis of glycogen, triglycerides, and protein. Insulin exerts its effects by suppressing the formation of cyclic AMP.

Insulin is unique, with respect to its effects on glucose uptake and a deficiency results in diabetes mellitus.

Insulin secretion is regulated by the integration of nutrient and neuro-hormonal signals (Pipeleers 1986, 1987). The nutrient signal is mediated locally at the level of the B cell while the neuro-hormonal signal constitutes a distal control mechanism. Increases in circulating levels of glucose or amino acids will cause an increase in insulin secretion. The islet cell response to circulating substrates is believed to be adequate to control plasma glucose levels at rest.

The integration of neuro-hormonal mechanisms in regulation of B cell secretion is only beginning to be understood but a variety of hormones and neurotransmitters have been identified to have effects on the B cell. Hormonally, the other pancreatic hormones have an effect on insulin secretion and are believed to play a role in paracrine regulation of insulin secretion, although this has yet to be proven (Samols et al 1986). Increases in glucagon and somatostatin will elicit increases and decreases, respectively, in the rate of insulin secretion (Pipeleers et al 1985). Epinephrine causes a decrease in insulin secretion (Pipeleers 1985, 1986) which is believed to underlie stress hyperglycemia (Halter et al 1984).

Electrical stimulation of the vagus nerve, which is the source of parasympathetic innervation to the islets, causes an increase in insulin secretion (Woods and Porte 1974; Miller 1981, Ahren et al 1986). Parasympathetic stimulation of the islets is thought to play a role in digestive and absorptive periods. The parasympathetic neurotransmitter, acetylcholine, is believed to exert its action directly on the B cell because the effects of vagal stimulation could be obviated by atropine,

a muscarinic receptor blocker, (Ahren and Taborsky 1986) and replicated by acetylcholine infusion (Iverson 1973).

Electrical stimulation of the splanchnic nerve, the main route of sympathetic innervation to the islets, has been observed to inhibit insulin secretion (Woods and Porte 1974, Miller 1981, Ahren et al 1986). Through the use of specific adrenergic blockade in the isolated perfused pancreas, it was concluded that insulin secretion was inhibited by an α_2 mechanism, while β_2 stimulation was capable of enhancing insulin secretion (Samols and Weir 1979). Until recently this double receptor adrenergic control of insulin secretion was accepted (Miller 1981, Smith and Davis 1983), however, this concept has been challenged. Ahren et al (1987) were unable to replicate the inhibition of insulin secretion observed during splanchnic nerve stimulation by infusing norepinephrine and concluded that splanchnic nerve stimulation might act through the release of neuropeptides and might not be adrenergically mediated. Schuit and Pipeleers (1986) investigated the effects of selective adrenergic agonists and antagonists in pure B cells and observed that insulin secretion was inhibited by α_2 receptor stimulation, and that β stimulation had no effect on insulin secretion which suggests that the previously observed elevation of insulin secretion might have been an indirect effect mediated via β adrenergic induced glucagon secretion.

Recently a new class of neurotransmitters has been discovered which is reshaping classical autonomic neurophysiology. It now appears that in addition to sympathetic and parasympathetic nervous systems, there is a third type of autonomic innervation referred to as peptidergic. Several neuropeptides have been identified within nerve fibers in the islets of Langerhans and have been demonstrated to effect insulin secretion (Ahren

et al 1986). Among these, vasoactive intestinal peptide (VIP) (Bishop et al 1980), cholecystokinin (CCK) (Rehfeld et al 1980), and gastrin-releasing polypeptide (GRP) (Moghimzadeh et al 1983) have been observed to increase insulin secretion. Galanin (McDonald et al 1985, Dunning et al 1986), pancreastatin (Tatemoto et al 1986), neuropeptide Y (NPY) (Pettersson et al 1987), and calcitonin gene related polypeptide (CGRP) (Pettersson et al 1986) have all been observed to inhibit insulin secretion. Of this group galanin bears further mention as it has been suggested to have a potency equivalent to somatostatin (Dunning et al 1986) and has been observed to cause hyperglycemia in dogs (McDonald et al 1986, 1987) and man (Bauer et al 1986). It should be noted that, while this list only includes neuropeptides which presently have been localized to the pancreas and have demonstrated effects, it cannot be considered to be final as there are neuropeptides being discovered with increasing frequency and it is only a matter of time before additional insulin regulatory neuropeptides are identified.

Glucagon

Glucagon is a 29 residue polypeptide hormone synthesized and secreted principally by the A cells of the islets of Langerhans. Glucagon is also secreted by A cells found in the fundus of the stomach (Orci et al 1983). There is a family of peptides secreted in the gastro-intestinal tract which bear a strong immunologic similarity to glucagon and are referred to as enteroglucagons (Holst 1983). These enteroglucagons are secreted by L cells in the small intestine and

include glicentin, oxyntomodulin, and glucagon related peptide. Recently it was observed that glucagon and the enteroglucagons are coded for on the same gene and are translated from identical RNA, but the differences occur during post-translational modification (Mojsov et al 1986).

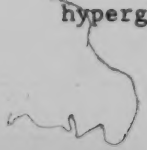
In the dog, glucagon has a half life of about five minutes and is principally cleared by the liver and the kidney (Jaspan et al 1981). The liver extracts about 25% of the glucagon passing through it or 28% of the total glucagon secreted, while the kidney extracts about 43% of the glucagon it sees or about 28% of the total glucagon secreted (Polonsky et al 1983a). In both the liver and kidney the clearance of glucagon was not saturable at high concentrations and was therefore suggested to be at least partially receptor independent. Recently it was observed that the liver is capable of rapidly trimming off three amino terminal residues, which are suggested to be essential for biological activity (Hagopian and Tager 1987). It was proposed that this metabolized glucagon might not be distinguished from native glucagon by immunoassay techniques and that the actual amount of glucagon cleared by the liver may as high as 42%, a level similar to that for hepatic insulin clearance.

Glucagon's effects appear to be limited to the liver and are in direct opposition to those of insulin. Glucagon stimulates glycogenolysis (Hems and Whitton 1980) and gluconeogenesis (Klaus-Friedman 1984) through a cyclic AMP dependent mechanism, causing an increase in glucose production. The effects of extrapancreatic glucagon and enteroglucagons appear normally to be limited to the gastrointestinal tract, although, following total pancreatectomy, normal

circulating glucagon levels (Vranic et al 1976, Holst et al 1983b) with identical biological potency (Doi et al 1979) of pancreatic glucagon have been observed due to secretion of glucagon from the A cells of the stomach.

Glucagon secretion is subject to the same two signal regulation as insulin secretion (Pipeleers 1986). The extrapancreatic glucagon and enteroglucagons appear to be regulated through digestive stimuli and there is little apparent innervation or response to circulating epinephrine (Lefebvre and Luyckx 1983). Locally, a drop in blood glucose or an increase in circulating amino acids, especially arginine, alanine, or glutamine, will cause an increase in pancreatic glucagon secretion (Pipeleers et al 1985).

As with the B cell, present understanding of neuro-hormonal regulation of A cells is limited. Both insulin and somatostatin are capable of inhibiting glucagon secretion and have been suggested to participate in paracrine regulation of glucagon secretion (Samols et al 1986). Because blood circulating through the islets of Langerhans encounters the B cells before reaching the A cells (Bonner-Weir and Orci 1982), the insulin induced inhibition of glucagon secretion has been suggested to play a strong role in modulating glucagon secretion. Furthermore, the withdrawal of this insulin induced inhibition, as occurs in diabetes, has been suggested to underlie diabetic hyperglucagonemia (Unger and Orci 1983). Exogenous somatostatin has been considered as a pharmacological method of reducing the diabetic hyperglucagonemia (Luyckx 1983). Epinephrine has been observed to increase glucagon secretion and has been suggested to play a role in mediating stress hyperglycemia (Halter et al 1984).



Vagus nerve stimulation has been observed to increase glucagon secretion (Woods and Porte 1974, Miller 1981, Palmer and Porte 1983, Ahren et al 1986). Contrary to earlier observations (Iverson 1973), Ahren and Taborsky (1986) were unable to inhibit the vagally induced increase in glucagon secretion with atropine but were able to completely inhibit it with hexamethonium, a ganglionic blocker. These results suggested that the A cells responded to neuropeptides released through vagal stimulation of intra-pancreatic ganglia.

Electrical stimulation of the splanchnic nerve has been observed to increase glucagon secretion (Woods and Porte 1974, Miller 1981, Palmer and Porte 1983, Ahren et al 1986). Through the use of receptor specific adrenergic agonists and antagonists, it was observed that both α_2 and β_2 stimulation resulted in increases of glucagon secretion (Samols and Weir 1979). As with adrenergic regulation of the B cell, this was undisputed until quite recently when Ahren et al (1987) found that norepinephrine infused into the isolated perfused canine pancreas only partially reproduced the effects on glucagon secretion that were observed with splanchnic nerve stimulation. They concluded that the remainder was probably due to neuropeptides. When Pipeleers and Schuit (1986) examined the response of pure A cells to selective adrenergic stimulation, they observed that β_2 stimulation caused an increase in glucagon secretion and that α_2 stimulation had no effect. These observations suggest that the increased secretion of glucagon after α_2 stimulation is an indirect effect resulting from the reduced inhibitory influence of insulin.

As with insulin secretion, there are a number of neuropeptides which have been observed to influence the A cell (Ahren et al 1986).

Among these VIP (Bishop et al 1980), CCK (Rehfeld et al 1980), and GRP (Mohgimzadeh et al 1983) elevate glucagon secretion coincident with their stimulatory effect on insulin secretion suggesting a parasympathetic nature to their action. As yet there have been no demonstrated effects of NPY and CGRP on glucagon secretion. Galanin has been observed to increase glucagon levels in the pancreatic vein but this increase was only subtly observable arterially (Dunning et al 1986). However, other groups have concluded that galanin does not exert an effect on glucagon secretion (Bauer et al 1986, Manabe et al 1986, McDonald et al 1986).

The Sympathoadrenal System

Together the sympathetic nervous system (SNS) and the adrenal medulla comprise the sympathoadrenal system (SAS). These components must be considered together because their effects are exerted by interaction with α and/or β adrenergic receptors on target cells. Both norepinephrine, released synaptically from the SNS, and epinephrine, released into circulation from the adrenal medulla, are capable of stimulating α and β receptors and thereby eliciting identical effects. The specificity of origin exists because the enzyme which converts norepinephrine to epinephrine is found only in the adrenal medulla (Ungar 1983). The catecholamines have a very brief lifetime in circulation, approximately 20 seconds (Callingham and Barrand 1979). Of the norepinephrine released by a sympathetic nerve terminal, the majority will be re-uptaken by the neuron, and the remainder inactivated

on the post-synaptic membrane, or will diffuse out of the synaptic cleft into circulation. Once in the circulation, norepinephrine can be cleared by the liver and excreted in the bile or cleared by the kidney and secreted in the urine. The clearance of epinephrine occurs through similar mechanisms but is not subject to re-uptake and probably does not encounter the same degree of enzymatic inactivation, leaving clearance by the kidney and liver as the major route of catabolism.

Epinephrine and norepinephrine both stimulate α and β adrenergic receptors. Because α and β adrenoreceptors elicit a wide range of responses throughout the body, for the purposes of this review only those pertinent to glucoregulation will be discussed. At the liver both α and β stimulation appear to be potent in causing an increase in glycogenolysis and gluconeogenesis (Hems and Whitton 1980). β stimulation is thought to evoke these responses by the same cAMP dependent pathways as glucagon (Exton et al 1979) but the mechanism of action of α stimulation is not well understood. At the muscle, acting entirely through β adrenergic mechanisms, sympathetic stimulation causes a decrease in glucose uptake, and an increase in glycogenolysis, lactate production and β -oxidation of fats (Issekutz 1980b, Woods et al 1986). In adipose tissue, also acting through β adrenoreceptors, stimulation results in an increase in lipolysis which elevates circulating free fatty acid levels (Woods et al 1986). In the pancreas α adrenergic stimulation has been observed to cause a decrease in insulin and somatostatin and an increase in glucagon secretion, while β adrenergic stimulation has been observed to result in an increase in the secretion of all three hormones (Samols and Weir 1979, Miller et al 1981). It is important to note that, although epinephrine and norepinephrine are both

capable of eliciting any of the above responses, the SNS can specifically stimulate a discrete response whereas epinephrine elicits a general response. During exercise, epinephrine is generally secreted in response to glycemic and hypoxic stress while norepinephrine secretion is more closely correlated with hemodynamic parameters, such as control of heart rate and control of peripheral circulation through vasoconstriction (Galbo et al 1983). The secretion of both norepinephrine and epinephrine is apparently under direct CNS control. This is, of course, obvious for norepinephrine, as it is the peripheral mechanism for expressing activity of the sympathetic nervous system. Similarly, epinephrine secretion from the adrenal medulla does not respond to local factors but appears instead to be under hypothalamic control (Ungar and Phillips 1983, Axelrod and Resine 1984).

B. INTEGRATION OF GLUCOREGULATORY CONTROL MECHANISMS DURING EXERCISE

Exercise provides a unique challenge to the glucoregulatory system. It has been observed that the rate of glucose utilization by the musculature increases 3 fold during exercise in dogs (Vranic et al 1976) and humans (Wahren et al 1971). The remarkable aspect to glucoregulation during exercise is that this increase in glucose utilization is precisely temporally matched by an equal increase in glucose production by the liver. The net effect is that the flux of glucose from liver to muscle triples, but circulating glucose concentration remains relatively constant. During exercise, because of the increased glucose flux, it is essential that the glucose production must not be exceeded by glucose utilization because hypoglycemia would occur very quickly. The exact mechanism by which glucose production and utilization are so tightly coupled remains a mystery but the roles of the acute glucoregulators have been investigated. Sympathoadrenal activity and the secretion of insulin and glucagon all undergo changes during exercise but it is noteworthy that the capacity of these regulators to exert fine control in coupling glucose production and utilization has been questioned (Newsholme 1979). However, a well characterized response to exercise is observed and, if perturbed, may effect glucose kinetics. The general theory for their action during exercise is that a drop in insulin sensitizes the liver to the diabetogenic actions of glucagon and the SAS, and peripheral β adrenergic stimulation promotes glycogenolysis in muscle, suppresses muscle glucose uptake, and mobilizes free fatty acids as an alternate substrate. While there are few who would dispute this basic mechanism,

there is confusion concerning precise roles played and their integration especially regarding the apparent redundancy of the SAS and pancreatic responses at the liver and, as such, the research in this area will be considered in some detail. This review of acute glucoregulation during exercise will attempt to discuss the essentiality of the various responses.

Experimental Approaches to Exercise Glucoregulation

To investigate the integration of the three major acute glucoregulators, insulin, glucagon, and the SAS, the obvious approach is to manipulate the components individually. There are three major problems encountered in the study of this response. Firstly, as will be discussed in more detail later, the SAS effects the secretion of the pancreatic hormones and insulin and glucagon influence each other's secretion. These are direct effects and may be difficult to discern from indirect effects mediated through changes in blood glucose, which is a powerful modulator of all three regulators. The result is that it is very difficult to perturb the normal responses and retrieve meaningful information about the integration of the system. Another problem stems from the fact that the biological actions of these three major glucoregulators overlap in some cases and are directly antagonistic in others, so that even if a specific perturbation were achieved it is still difficult to interpret. Finally, because the glucoregulatory response to exercise involves the coordination of central and peripheral factors, an in vivo model is required. There are

a multitude of problems associated with investigating whole body responses which make interpretation of observations difficult.

Most of the research has been carried out in three models. While rats are economical and fairly well characterized, because of a low blood volume and difficulty in drawing blood, researchers have largely been limited to a pre- post experimental design. The rat has proved to be a good model for examining the extent of glycogenolysis, in both liver and muscle which are more difficult to examine in larger organisms. Humans have understandably received considerable attention, but there are several problems associated with this model. Although blood is accessible to serial sampling, due to ethical constraints it must be obtained peripherally. The same limitations concern infusion of drugs and there is evidence which indicates that the route of delivery with respect to the liver plays an important role in determining the effect of these agents. The dog is an excellent model for examining glucoregulatory integration, as it is large enough for serial blood sampling and is not subject to the same ethical constraints as humans. This has allowed acute surgical intervention, including pancreatectomy, adrenalectomy, catheterization of the hepatic portal vein and artery. An additional advantage of the dog model over humans is that it is much easier to control for external factors, such as diet, training, and cooperation. A degree of caution is warranted when comparing observations obtained from different species, as the research in this area has not yet reached the point where any animal model can truly be ~~considered~~ well characterized.

The study of glucoregulation has been greatly facilitated by the development of several experimental techniques. A primary requirement

for the study of glucoregulation was a method to observe glucose production and utilization. Two general approaches have been used to measure these processes. The simplest and most direct method is to measure arterial-venous differences across the liver or muscle. A second and more powerful method is the use of radioactive tracers to study substrate kinetics (Hetenyi et al 1983, Wasserman and Vranic 1986). Briefly, this technique involves infusing a radiolabeled substrate at a known rate and measuring the specific activity of sampled blood. By expressing the measured radioactivity in different ways (i.e. relative to infusion rate, sample volume, or substrate concentration) the rate of appearance, rate of disappearance, and metabolic clearance of the substrate in question can be calculated. A number of substrates have been examined by this technique including glucose, lactate, free fatty acids. The use of these various tracers has been principally employed in dogs and humans and has been invaluable to the study of substrate kinetics at rest and exercise.

Several experimental designs have been employed to perturb the normal glucoregulatory system. Somatostatin has been widely used to control or suppress the secretion of insulin and glucagon by the endocrine pancreas. Somatostatin, which is secreted by the D cells of the islets of Langerhans, the gut, and also in the CNS, is a potent inhibitor of both insulin and glucagon secretion and has been suggested to play a role in their physiologic control as will be discussed in more detail later. When infused in sufficient concentrations, somatostatin suppresses insulin and glucagon secretion and does not directly effect carbohydrate metabolism (Byrne et al 1977). Somatostatin has been infused alone or in conjunction with insulin and/or glucagon to study

effects on glucose kinetics. The infusion of somatostatin and exogenous insulin and glucagon, referred to as an "islet clamp", has made it possible to control insulin and glucagon levels. This has been an important contribution because it has allowed researchers to study the effects of various treatments on glucose kinetics without complication by changes in insulin and glucagon. Mannoheptulose, which is a specific inhibitor of insulin secretion, has been used to depress insulin levels without effect on glucagon secretion. Pancreatectomy has also been employed to eliminate the secretion of pancreatic hormones. Basal glucagon levels are not effected by pancreatectomy because of glucagon secretion from the stomach, while insulin secretion is abolished. A final method of controlling insulin levels involves simply infusing insulin peripherally or portally or injecting long-acting insulin subcutaneously.

It is understandably more difficult to experimentally control the sympathoadrenal aspect of glucoregulation. Because of the disperse nature of the SAS, the most frequently used approach involves blocking α adrenoreceptors with phentolamine or β adrenoreceptors with propranolol. Because of the central role of α adrenergic mediated peripheral vasoconstriction in the general response to exercise, β adrenergic blockade has been used widely in exercise studies while α adrenergic blockade has only rarely been attempted. Infusion with either α or β adrenergic blockers causes a marked elevation of circulating epinephrine and norepinephrine (Galbo et al 1976,1977) which may lead to misinterpretation of observations if both blockers are not used in concert. The use of α and β adrenergic blockers is also complicated because α adrenoreceptors are suggested to be involved in the inhibition

of insulin secretion during exercise and both α and β adrenoreceptors could potentially be involved in the elevation of glucagon secretion (Miller et al 1981, Palmer and Porte 1983). Therefore a reasonable possibility exists that the observed effects of α or β adrenergic blockade may arise indirectly. Other approaches to the study of sympathoadrenal involvement in glucoregulation include infusing epinephrine and adrenodemodulation. Recently Cryer and co-workers (Hoelzer et al 1986 a,b) have combined an islet clamp with α and β adrenergic blockade during exercise and this design appears to have great potential.

Role of Insulin

Extra-Hepatic Effects

There is considerable confusion regarding the extra-hepatic role of insulin during exercise (Wasserman and Vranic 1986, Hollosy 1986). Insulin's most widely known effect is, of course, the facilitation of glucose uptake in the periphery. Paradoxically, during the period where glucose uptake by muscle increases greatly, the circulating insulin concentration drops markedly (Cochran et al 1966, Vranic and Wrenshall 1969, Vranic et al 1976). Kawamori and Vranic (1977) found that, if depancreatized dogs were given subcutaneous long-acting insulin prior to exercise, the dogs became hypoglycemic after the enhanced mobilization of the injected insulin resulted in marked, though transient, hyperinsulinemia. Utilizing a tracer methodology they were able to

conclude that the glucose uptake by the muscle followed the normal course and therefore the observed hypoglycemia resulted from a decrease in hepatic glucose production. Issekutz (1980) used mannoheptulose to cause the exercise-induced decrease in insulin secretion to fall still lower and observed no impact on metabolic clearance of glucose, and concluded that insulin's role in exercise was limited to the liver and that the exercise induced elevation of glucose uptake was a result of metabolic demand. However, Vranic and co-workers (1976) observed that depancreatized dogs, from which insulin had been withdrawn for 48 hours, were incapable of increasing the metabolic clearance rate of glucose during exercise. Unfortunately, this observation is complicated by the fact that the plasma glucose concentration in these dogs rose to approximately 300 mg/dl and the actual rate of glucose utilization was comparable to the controls, which has cast doubts upon its physiological significance. Equally problematic, with regard to physiological significance, is the observation that exercise during sustained marked hyperinsulinemia, approximately 115 μ U/ml, slightly increased glucose clearance across exercising musculature above exercise alone (Felig et al 1981). These experimental results appear to indicate that there is at least some degree of insulin independence to the exercise-induced increase in the rate of glucose utilization.

Several explanations have been suggested to explain this paradox. Because blood flow to muscle is greatly enhanced by activity, it has been postulated that the muscle "sees" more insulin during exercise despite the drop in actual concentration (Vranic et al 1976). It has also been suggested that the exercising musculature binds insulin with higher affinity, thereby adapting to decreases in insulin concentration

(Berger et al 1978, Kalant et al 1978). It is now well known that muscle contraction, whether neurally or electrically initiated, is capable of causing an increase in glucose permeability independent of, and additive to, insulin (Holloszy et al 1986). This increase in glucose permeability is also independent of the force, or energy requirement, of contraction, which suggests that it does not arise through alteration of concentration gradients evoked by an increase in glucose metabolism and cannot be expected to participate in meeting the metabolic demand of the working muscle with any degree of precision. It has also been suggested that the intramuscular glucose-6-phosphate concentration may play a role in coupling glucose transport with metabolic demand in active muscle (Newsholme 1979, Issekutz 1984). Because there are no acceptable answers to this problem at present, it may be best to accept that there is a presently undefined interaction between metabolic demand, circulating insulin concentration, and contraction induced glucose permeability which allows the exercising muscle to increase glucose utilization.

Hepatic Effects

It has been suggested that the decrease in insulin secretion, which occurs with the onset of exercise, plays an important role in sensitizing the liver to the influence of glucagon and SAS activity (Wasserman and Vranic 1986). Cherrington et al (1978) observed in resting conscious dogs that hepatic glucose production doubled during somatostatin infusion, which inhibited both insulin and glucagon secretion, if basal glucagon levels were maintained through infusion.

This increase in glucose production was due to both glycogenolysis and gluconeogenesis, although glycogenolysis was predominant. These observations were confirmed by Lickley et al (1979) who noted similar effects of relative hypoinsulinemia on hepatic glucose production, but found that if relative hyperinsulinemia was evoked by infusing insulin with somatostatin instead of glucagon, there was no significant difference compared to somatostatin alone with respect to glucose production. Sacca et al (1979) observed that intravenous insulin injection resulted in hypoglycemia through stimulation of glucose uptake and suppression of glucose production. The results of these studies are in accord with insulin's direct antagonism to glucagon at the liver.

Several researchers have perturbed the normal B cell response to exercise in an attempt to further elucidate insulin's role in exercise glucoregulation. It has been observed in exercising dogs that if insulin secretion is specifically suppressed below normal exercise levels by infusion of mannoheptulose, hepatic glucose production rises threefold above normal exercise levels, indicating that insulin may play more than a passive role in the control of glycemia (Issekutz 1980). Several groups have investigated the glucoregulatory response to exercise in the absence of a decrease in insulin achieved via insulin or islet clamp or by creating a hyperinsulinemic state by infusing exogenous insulin. When depancreatized dogs exercised after receiving subcutaneous injections of long-acting insulin they became hypoglycemic (Kawamori and Vranic 1977). At the initiation of exercise, circulating immunoreactive insulin (IRI) was greatly elevated, reaching over 100 μ U/ml, presumably due to an increase in the mobilization of the subcutaneous insulin. An analysis of the glucose kinetics revealed that

the primary defect underlying the hypoglycemia was a marked diminution of the normal exercise induced increment in hepatic glucose production. In normal humans, when exercise induced changes in pancreatic hormone secretion were prevented by a basal glucose infusion, splanchnic glucose production still occurred despite the "clamping" of insulin and glucagon (Felig and Wahren 1979). If, however, this protocol was repeated with concurrent insulin infusion, a hyperinsulinemic state occurred with plasma IRI reaching 100 μ U/ml (fivefold greater than basal IRI) and splanchnic glucose production did not increase in response to exercise. When plasma glucose was rigorously clamped during low intensity exercise (heart rate = 100 bpm) in humans, it was observed that there was fivefold greater requirement of exogenous glucose when IRI levels were maintained at 20 μ U/ml (Martin et al 1981). Unfortunately, this study does not indicate whether the increase in glucose demand arose from a decrease in glucose production or an increase in glucose utilization. In a different study (Wolfe et al 1986), where insulin was clamped at a similar level, in humans exercising at a similar low intensity, hypoglycemia occurred and was due to a disproportionate increase in glucose utilization. However, these observations may be a product of the very low exercise intensities employed, which may not constitute a sufficient stimulus for complete blood flow shunting allowing insulin to exert its hypoglycemic actions on relatively inactive muscle.

In summary, there is evidence that insulin plays an important role during exercise. It appears that the decrement in insulin secretion does not impair glucose uptake by the exercising musculature. Hepatic glucose production appears to be potently modulated by insulin. While hypoinsulinemia per se potentiates the response of the liver to glucagon

and sympathoadrenal activity, Issekutz's (1980) observations strongly suggest that the degree of hypoinsulinemia may have a regulatory as opposed to merely permissive capacity. However, the recent studies of Cryer and associates (Hoelzer et al 1986a, b, Tuttle et al 1987) suggests that the pancreatic response to exercise is redundant with the SAS at the liver, indicating that insulin does not play a crucial role during exercise, as will be discussed in detail later. While the insulin response to exercise may be redundant, the above mentioned research provides ample evidence that a serious perturbation of the normal insulin response may directly effect glucose kinetics.

Role of Glucagon and the Sympathoadrenal System in Exercise

Extra-Hepatic Effects

The SAS has considerable impact on extrahepatic aspects of glucoregulation, while glucagon's effects are believed to be confined to the liver. In resting dogs, epinephrine infusion has been observed to result in increased muscle glycogenolysis and lactate production, while decreasing metabolic clearance of plasma glucose and decreasing the percentage of lactate originating from plasma glucose (Issekutz 1985). Attempting to control for indirect effects of epinephrine mediated through effects on the pancreatic hormones, Gray et al (1980) infused epinephrine in the presence and absence of somatostatin and observed that epinephrine elevated circulating free fatty acid levels and decreased the metabolic clearance of plasma glucose independently of

glucagon. Cherrington et al (1984) infused epinephrine in the presence and absence of a somatostatin-mediated islet clamp and observed similar effects on the metabolic clearance of plasma glucose and increases in circulating lactate and alanine levels while pancreatic hormone levels were held constant. Sacca et al (1979) observed that an epinephrine infusion was capable of obviating insulin induced increases in glucose uptake.

During exercise the role of the SAS was initially explored through the administration of β blockers. It was observed in exercising dogs that β blockade decreased the rate of mobilization of free fatty acids and increased the metabolic clearance of plasma glucose and the percentage of lactate produced from plasma glucose (Issekutz 1978). In later work, Issekutz (1984) observed that β blockade increased the metabolic clearance of lactate and decreased the rate of lactate production and circulating concentrations. In studies on rats, Winder and co-workers have observed that adrenalectomy reduces muscle glycogenolysis during moderate (Winder et al 1986) and intense exercise (Marker et al 1986) and that during moderate exercise the extent of muscle glycogenolysis was dependent on the magnitude of epinephrine infusion (Arnall et al 1986).

This body of research has provided convincing evidence of the important role played by β adrenergic stimulation in mobilization and utilization of extra-hepatic substrates during rest and exercise. The observations of Winder and co-workers (Winder et al 1986, Marker et al 1986, Arnall et al 1986) are interesting in that they identify epinephrine as being essential to muscle glycogenolysis. While the muscles per se do not receive a great deal of SNS innervation, the blood

vessels permeating them do and it has been suggested that the spatial arrangement between muscle tissue and vasculature is intimate enough that SNS stimulation of the vasculature may "spill over" to the muscles (Woods et al 1986). This would be in contradiction to Winder's observations and it would be interesting to see this inconsistency explored in dogs or humans.

Hepatic Effects

Both glucagon and the SAS are capable of elevating hepatic glucose production at rest. However, it is important to note that on a molar basis, glucagon is approximately ten times as potent as epinephrine (Sokal et al 1964). As previously noted, relative hyperglucagonemia appears to be an adequate stimulant of hepatic glucose production in the resting dog (Cherrington et al 1978, Lickley et al 1979). At rest epinephrine infusion has been observed to elevate the rate of hepatic glucose production in dogs (Issekutz et al 1985) and humans (Cryer et al 1980). Concurrent infusion of epinephrine and somatostatin indicated that this elevation was not mediated indirectly through enhanced glucagon secretion (Gray et al 1980). These observations were confirmed in a later study which employed an islet clamp to maintain pancreatic hormones at basal levels (Cherrington et al 1984). This study went on to indicate that the increased rate of hepatic glucose production resulted from elevations of both glycogenolysis and gluconeogenesis, although glycogenolysis dominated. Cherrington and co-workers concluded that in contrast to the stimulation of glycogenolysis, the gluconeogenesis was not actually stimulated but was simply responding to

an increased supply of gluconeogenic substrates from the periphery. While both glucagon and epinephrine are capable of elevating plasma glucose levels, if infused together their hyperglycemic effect is greater than the sum of their individual actions, thus indicating a degree of synergism (Eigler et al 1979). As well, both glucagon and epinephrine have been observed to overcome insulin induced suppression of hepatic glucose production (Sacca et al 1979).

During exercise a steady rise in immunoreactive glucagon (IRG) is a common observation in dogs (Vranic et al 1976, Wasserman et al 1984), rats (Luyckx and Lefebvre 1974), and humans (Bottger et al 1972). Vranic et al (1976) noted that depancreatized dogs which had normal basal IRG originating from extrapancreatic sources, and which received normal insulin via intraportal infusion, were capable of increasing their hepatic glucose production despite the absence of an exercise induced increment in IRG. The conclusions from this study were that the regulation of extrapancreatic glucagon secretion differs from pancreatic glucagon and that an increase in IRG may not be essential for hepatic glucose production. If glucagon secretion was suppressed during exercise by somatostatin infusion, dogs became hypoglycemic due to an inadequate rate of hepatic glucose production (Issekutz and Vranic 1980). The exercise response could be normalized if glucagon was co-infused with the somatostatin. In exercising rats, it was observed that the infusion of antigen stripped anti-glucagon antibodies to the portal circulation decreased the extent of hepatic glycogenolysis (Richter et al 1981).

Normal exercise in humans (Galbo et al 1976, 1977) and dogs (Wasserman et al 1984) induces a steady elevation in circulating levels

of both epinephrine and norepinephrine. During exercise in dogs, Issekutz (1978, 1984) observed that hepatic glucose production was preserved during β blockade. The mechanism by which hepatic glucose production was maintained during β blockade was not established by these studies but it was suggested that, in the absence of β stimulation, hypoinsulinemia, α adrenergic mechanisms, and metabolic factors may have been capable of supporting hepatic glucose production. However, because of the increase in metabolic clearance of plasma glucose during β blockade, the rate of glucose utilization greatly increased and hypoglycemia resulted. It is important to note that, in the absence of β adrenergic stimulation, hepatic glucose production was incapable of meeting demand. In a later study Issekutz (1985) observed that epinephrine infusion during exercise resulted in a hyperglycemia due to a decrease in glucose utilization because glucose production did not rise. The absence of an elevation of hepatic glucose production was attributed to the inhibitory effect of hyperglycemia on hepatic glycogenolysis. However, when resting dogs were infused with similar doses of epinephrine, a greater degree of hyperglycemia was observed, although the rate of glucose production was not as high (Gray et al 1980) which may indicate that there is some limit to the extent of epinephrine stimulated glucose production or that the action of epinephrine on the liver may be modulated by exercise. In studies in exercising rats, where it was possible to measure the extent of liver glycogenolysis, it was observed that adrenalectomy had no impact on liver glycogenolysis (Winder et al 1986, Marker et al 1986) and that infused epinephrine was also without effect (Arnall et al 1986). The absence of effect of epinephrine infusion and adrenalectomy strongly

suggest that the SNS component of the SAS is active in stimulating hepatic glycogenolysis.

In summary, circulating levels of glucagon, norepinephrine, and epinephrine all rise in response to exercise. The question of which one, or ones, are responsible for the elevation of hepatic glucose production during exercise remains, although all are capable of the task at rest. While hypoglucagonemia decreases hepatic glucose production, basal levels appear to be sufficient for glucose production to proceed unimpaired. There is no apparent effect of epinephrine or β blockade, although one exists at rest. As will be referred to in detail in the next section, combined α β blockade is also without effect on glucose kinetics during exercise (Hoelzer et al 1986a, b). The only conclusion which can be drawn is that glucose production can be elevated by all of the above in a redundant manner, such that if the exercise induced increment of one is prevented in isolation, the others can make up the difference. From a physiological perspective, it is understandable that the maintenance of such a crucial substrate as glucose should be subject to redundant control.

Redundancy of Pancreatic and Sympathetic Responses to Exercise

Several studies have suggested that there is a considerable redundancy between the action of glucagon and the SAS in the prevention of hypoglycemia. In human studies, Cryer and co-workers (1984) examined the return to normoglycemia from insulin induced hypoglycemia and glucose induced hyperglycemia. It was observed that glucagon

suppression, adrenalectomy, and combined $\alpha \beta$ adrenergic blockade had little effect individually, but if glucagon suppression was combined with sympathetic perturbation the recovery from hypoglycemia was prolonged and the recovery from hyperglycemia resulted in marked rebound hypoglycemia thus indicating a redundant but essential role for glucagon and the SAS in resting glucoregulation.

As previously mentioned, if glucagon secretion was suppressed during exercise by somatostatin infusion, dogs became hypoglycemic due to an inadequate rate of hepatic glucose production (Issekutz and Vranic 1980). The marked decrease in the metabolic clearance of glucagon and increases in lactate and free fatty acid levels were consistent with an increase in sympathoadrenal activity, which unfortunately was not measured. The overlap in biological effect between glucagon and sympathoadrenal activity at the liver and peripheral effects of β adrenergic stimulation were well established by this time and the authors suggested that a catecholamine response probably played a role in their observations. Wasserman et al (1984) built on the work of Issekutz and Vranic (1980) and added measures of catecholamines to the previous study. They observed similar responses in glucose kinetics, lactate, and free fatty acids and noted a fourfold increase in epinephrine over control exercise. If glucose was infused along with somatostatin so that the rate of total glucose appearance was equivalent to control exercise, epinephrine and lactate responses returned to control levels indicating that they were a response to hypoglycemia. They concluded that in the presence of hypoinsulinemia IRG was responsible for 70% of the increase in hepatic glucose production and that the maximal catecholamine contribution was 25%. A problem with

this study is that glucagon levels were suppressed well below basal secretion and there is evidence (Vranic et al 1976) to suggest that basal glucagon levels may be adequate to stimulate hepatic glucose production during exercise in dogs. It is important to note that the dose of somatostatin infused ($0.5 \mu\text{g/kg-min}$) did not suppress insulin secretion below the normal exercise induced decrement, which makes this study particularly interesting because glucagon secretion was apparently perturbed in isolation. This study strongly suggests that glucagon plays an important role in the elevation of hepatic glucose production during exercise and identifies epinephrine's role in responding to hypoglycemic emergencies. Recently Wasserman and co-workers (1987) have reported a novel experimental approach wherein both insulin and glucagon were clamped during exercise in dogs. Although a full report of their observations is not yet available the insulin clamp appears to have greatly diminished hepatic glucose production and gluconeogenesis. From these observations they concluded that the normal pancreatic response to exercise is essential for hepatic glucose production during exercise. Until the full report is made, it would appear to be unwise to base conclusions on these observations although they should be recognized.

Recently, an excellent experimental design has been employed by Cryer and co-workers (Hoelzer et al 1986a,b) in which the glucose kinetics and hormonal responses to exercise in humans were observed in the presence of an islet clamp and/or combined α and β adrenergic blockade (Hoelzer et al 1986a) or adrenalectomy (Hoelzer et al 1986b). When the islet clamp was employed in isolation, there was no impact on glucose kinetics or catecholamine responses (Hoelzer et al 1986a). The combined α and β adrenergic blockade resulted in an initial transient

increase in the rate of glucose utilization, which resulted in a lowered plasma glucose which was maintained until the end of exercise. When the islet clamp and adrenergic blockade were concurrently imposed, a marked decrease occurred in glucose production while glucose utilization was unaffected resulting in hypoglycemia. Adrenalectomized patients displayed a normal exercise response and the islet blockade was without effect (Hoelzer et al 1986b). Very recently, this same group has observed that, if the insulin response is prevented and glucagon is free to change, the glucose kinetics were similar irrespective of adrenergic blockade. These observations suggest that a strong role in the glucoregulatory response to exercise is played by neural control of the liver and it is redundant with pancreatic hormone effects.

It is interesting that the isolated islet clamp had no apparent effects in normal and adrenalectomized humans. These observations are in opposition to those of Wasserman and co-workers (1987), who firmly concluded that the normal pancreatic response to exercise was essential for glucose kinetics to proceed unimpaired during exercise in dogs. This contradiction may be due to species differences between humans and dogs. A study which clamped the pancreatic hormones at similar levels, during exercise in humans, observed a marked rise in epinephrine levels and hypoglycemia (Wolfe et al 1986). In this study, however, hypoglycemia resulted from elevated glucose utilization, not suppression of glucose production. This may have occurred because the intensity of the exercise was very low with the heart rate maintained at about 110 bpm, which may have allowed greater impact of insulin on inactive tissues. It is important to emphasize that the marked rise in epinephrine observed by Wasserman (1984) was a response to hypoglycemia.

and not a direct effect of the glucagon suppression. Wolfe and co-workers (1986) probably observed a similar effect. It then follows that some mechanism was responsible for maintaining normoglycemia in Cryer's islet clamp studies. It may be possible that basal glucagon levels were sufficient when augmented by hepatic innervation to stimulate glucose production. It is possible that elevated neural stimulation of the liver might be masked by the normal norepinephrine response to exercise. However, it is also possible that the islet clamp was not actually achieved. During the islet clamp, insulin and glucagon were infused via a peripheral vein which may have resulted in relative hypoinsulinemia at the liver. It would be interesting to see these studies repeated in the dog, where the islet clamp could more confidently be achieved.

Both α and β adrenergic blockade, individually or in concert, cause circulating levels of catecholamines to markedly increase (Galbo et al 1976, 1977, Simonson et al 1984), as they did in these studies (Hoelzer et al 1986a, b), and may increase to the extent that the blockade is overcome. There are several observations from this study that are incompatible with complete adrenergic blockade. Firstly, insulin levels followed their normal course when it has been shown that the exercise induced decrement in insulin can be obviated by α adrenergic blockade (Luyckx and Lefebvre 1974, Galbo et al 1977, Jarhult and Holst 1979, Simonson et al 1984). Glucagon levels actually exceeded the normal exercise-induced increment when previous studies have indicated that glucagon suppression occurs with α (Harvey et al 1974) or β (Luyckx and Lefebvre 1974, Simonson et al 1984) or combined α and β adrenergic blockade (Simonson et al 1984). Finally, plasma lactate levels followed

the normal course, although Issekutz (1978, 1984) has shown that lactate production is dependent upon β adrenergic stimulation. While these observations strongly suggest that the adrenergic blockade was not successful, the suppression of free fatty acid levels, depression of heart rate and blood pressure, and the subjective increased difficulty of the exercise all suggest that at least some degree of adrenergic blockade was achieved.

In summary, it would appear that there is a degree of redundancy in the roles played by the pancreatic and SAS responses during exercise in the control of hepatic glucose production. However, because of the SAS's important effect on peripheral substrate mobilization and metabolism, it can be considered to be the primary defence against hypoglycemia. This should not be interpreted as an indication that, during normal exercise, the pancreatic response is impotent. While it appears that, if clamped at "basal" levels, the glucoregulatory response to exercise is unimpaired in humans, it is important to recall that glucose kinetics can be disrupted via hyperinsulinemia (Kawamori et al 1977, Felig and Wahren 1979, Wasserman et al 1987) or hypoglucagonemia (Issekutz and Vranic 1980, Wasserman et al 1984). At this point in time it is difficult to reach a conclusion regarding the individual roles of the SNS and epinephrine. The adrenalectomy studies are conflicting in that during exercise in adrenalectomized rats, muscle glycogenolysis was totally dependent upon epinephrine (Arnall et al 1986, Marker et al 1986, Winder et al 1986), while in the humans there was no apparent impact of adrenalectomy on lactate or free fatty acid levels (Hoelzer et al 1986a, b). Once again it is difficult to draw any firm conclusions

because of the very real possibility of species differences between rats and man.

The integration of glucoregulatory mechanisms has been schematically summarized in figures 16 to 19. Glucoregulation can be considered to be a balance between glucose utilization and glucose production. Glucose utilization can be considered to be the difference between the energy requirement of the periphery and the extent to which this requirement is met by fuels other than blood glucose, such as free fatty acids and intramuscular fat and glycogen depots. Glucose production can be considered to be a product of the balance between the impact of inhibitory (insulin) and stimulatory (glucagon and the SAS) mechanisms at the liver. During post-absorptive rest (see Fig. 16), normoglycemia appears to be maintained by the islets of Langerhans via feedback from plasma glucose concentrations, with only basal activity of the SAS.

During normal exercise (see Fig. 17), normoglycemia is maintained by the matching of glucose utilization and glucose production. It appears that the hypothalamus becomes more sensitive to plasma glucose levels or to glucose balance, or some integration of both, and coordinates the SAS and pancreatic responses. The increased energy demand of the active musculature is partially met by an increase in glucose utilization. The remainder of the energy requirement is met by elevated muscle glycogenolysis and free fatty acid mobilization mediated by β adrenergic stimulation. Glucose production is elevated by a decrease in insulin secretion and an increase in glucagon secretion. This pancreatic response is probably augmented by α and β adrenergic

stimulation of the liver by both epinephrine from the adrenal medulla and norepinephrine from the sympathetic nervous system.

If, for a variety of reasons including hyperinsulinemia or hypoglucagonemia, this normal response is disrupted and glucose production is incapable of matching glucose demand, hypoglycemia will ensue (see Fig. 18). Wasserman and co-workers (1984) have suggested that the set-point glucose concentration for the onset of the epinephrine response to hypoglycemia during exercise, about 80 mg/dl, is elevated relative to rest, about 60 mg/dl. During a state of high substrate flux, as is observed with glucose during exercise, any inequality of production and utilization will have a greatly magnified impact relative to basal flux. This has been suggested to be the reason underlying the heightened sensitivity to hypoglycemia during exercise. The response to exercise-induced hypoglycemia is schematically depicted in figure 19. The hypothalamus strongly stimulates the adrenal medulla and the resulting rise in circulating epinephrine decreases glucose utilization and increases glucose production. Glucose utilization is suppressed via β adrenergic stimulated muscle glycogenolysis and free fatty acid mobilization. At the liver, epinephrine enhances glucose production directly through adrenergic stimulation, and indirectly through stimulation of glucagon release. It is important to note that in recovery from hypoglycemia glucose production must not merely meet utilization, it must exceed it.

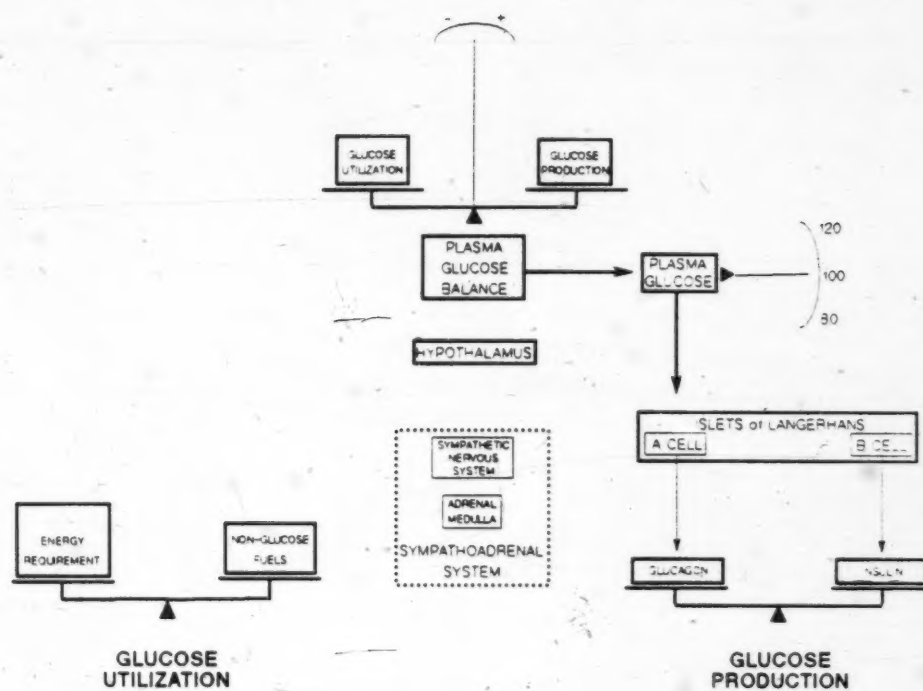


Fig. 16

Schematic representation of glucoregulatory control during post-absorptive rest

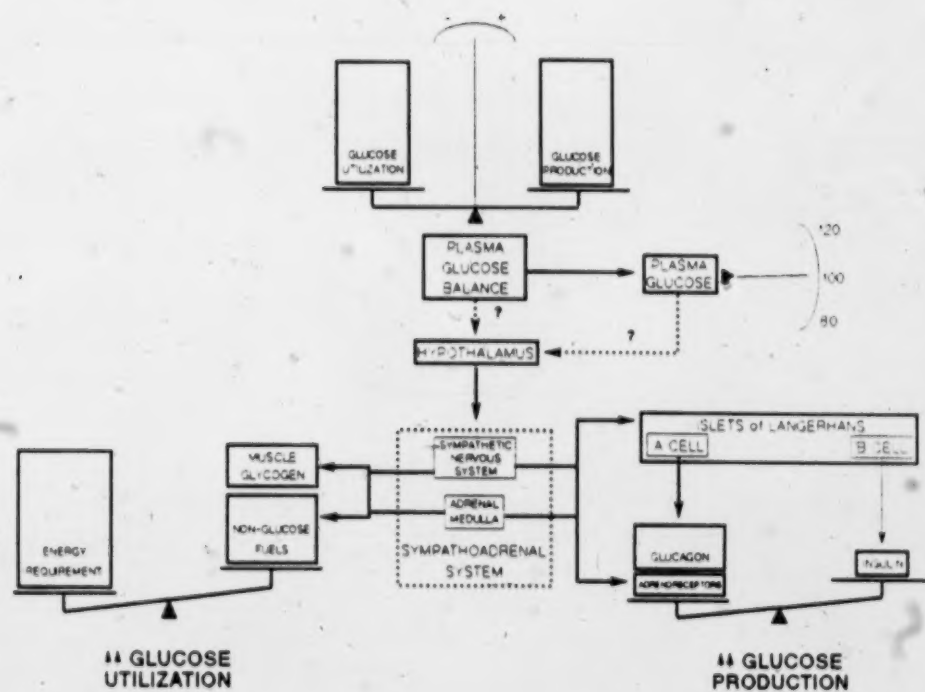


Fig. 17

Schematic representation of glucoregulatory control during exercise

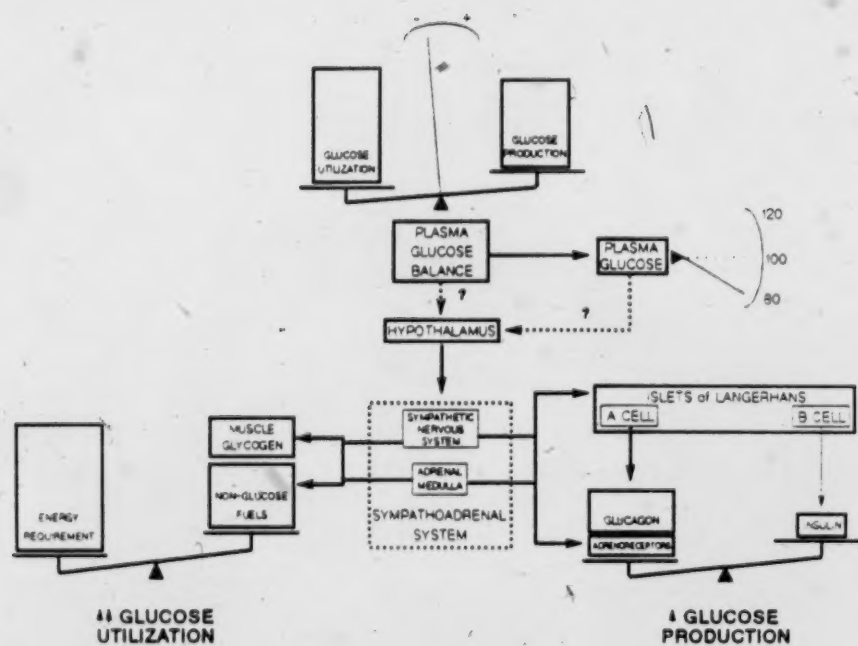


Fig. 18

Schematic representation of glucoregulatory imbalance (glucose utilization exceeds glucose production) creating a hypoglycemic state during exercise

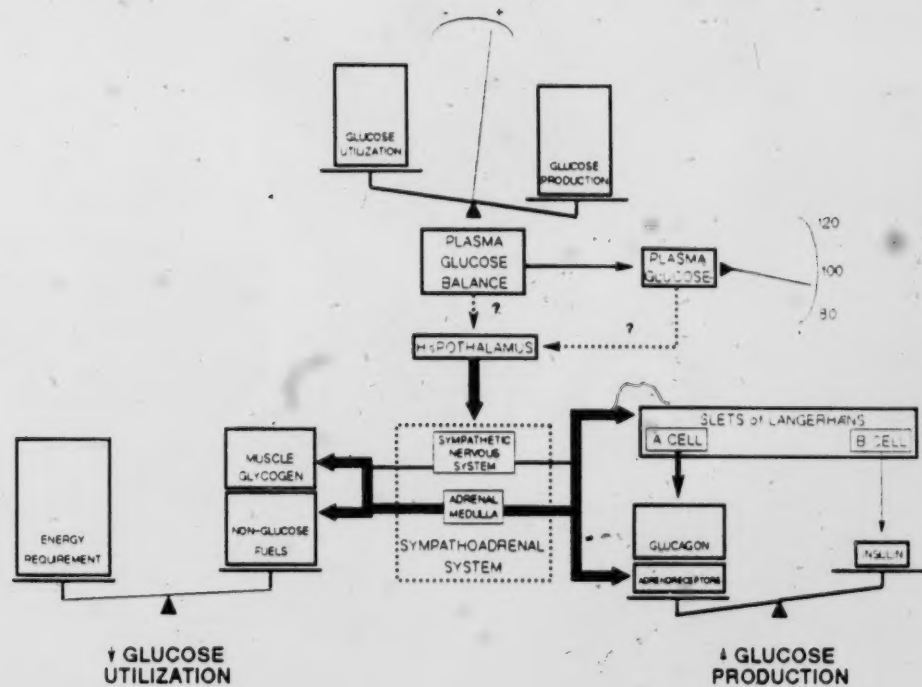


Fig. 19

Schematic representation of glucoregulatory control mechanisms for recovery from hypoglycemic distress during exercise

C. ISLET CELL TRANSPLANTATION

Transplantation of the pancreas has been considered as a therapy for diabetes since the glucoregulatory role of the pancreas was first identified. Whole or segmental pancreas transplantation has been attempted with limited success (Sutherland et al 1984). Many of the problems with pancreas transplantation are due to the grafting of exocrine tissue in addition to the islet cells. The grafted exocrine tissue still produces digestive enzymes which may digest islet cells. Because the exocrine portion of the pancreas makes no beneficial contribution to the function or survival of the graft, researchers have attempted to transplant only the islets of Langerhans, which constitute only about 1% of the total pancreatic tissue.

The initial problem in islet cell transplantation was the isolation of the islet cells from the remainder of the pancreatic tissue. Because the islets are relatively few in number and disperse in location within the pancreas, the isolation of viable islets in high yield is still a major obstacle to transplantation. A major breakthrough was achieved when Moskalewski (1965) used collagenase to isolate islet cells from chopped guinea pig pancreas. This method provided a basis for Lacy and Kostinovsky (1967), who improved upon it by disrupting the exocrine tissue through intraductal distension followed by mincing and collagenase digestion. They were then able to isolate approximately 300 islets per rat from the remaining pancreatic tissue through sucrose gradient centrifugation. While layered ficoll has been substituted for sucrose in the centrifugation step (Scharp et al 1973) and further

isolation has been achieved by hand-picking in the final stage (Finke et al 1979), the technique of Lacy and Kostianovsky forms the basis of modern islet cell isolation.

With the availability of isolated islets, the technical aspects of transplantation could now be investigated. Because of the availability of inbred strains, initial experiments were conducted in rodents. Because multiple donors were required for a single recipient, syngeneic rodents were essential for the early work as they avoided the complication of immunological problems. The first attempt at islet cell transplantation in rodents used outbred diabetic rats and achieved temporary amelioration before rejection occurred (Younoszai 1970). Ballinger and Lacy (1972) were the first to observe sustained function of grafted islets after transplanting 400-600 islets intraperitoneally. Normalization of blood glucose was observed after intraperitoneal transplantation of a greater number of islets in rats (Reckard et al 1973).

After successful islet cell transplantation was observed to be possible researchers now had to deal with two further issues - How many islets are required and where should they be placed. Kemp et al (1973) observed that intraportal placement of the grafted islets was more efficient than intraperitoneal transplantation. The portal system has since become the preferred site for transplantation in rats, although virtually every other site has been attempted (Sutherland et al 1984). With regard to the number of islets required for successful transplantation, with increasing islet number the latent period between transplantation and diabetic amelioration and glucose tolerance improve (Violettes et al 1979). When transplantation was successful, diabetic

rats were generally normalized with the notable exception of small differences in glucose tolerance which has been attributed to the relocation of the islets (Pipeleers et al 1978), denervation (Berthoud et al 1980), disruption of the enteroinsular axis (Siegal et al 1980a, b), or to diminished islet mass (Scharp et al 1982). Work in the rodent model is continuing, examining the potential of fetal and neonatal islet transplantation as well as investigating the immunological aspects of transplantation.

The rodent studies have suggested that there is great potential for clinical islet cell transplantation. When researchers began to investigate islet transplantation in large animal models they were confronted with two major problems. Because syngeneic multiple donor transplantation was no longer possible, they were forced to use an autografted model to avoid immunogenic complications. The second problem was encountered in the large animal pancreas which is more fibrous and compact than the rodent pancreas, which decreased yield (Scharp et al 1980). The initial attempts at "islet" transplantation avoided the problem of low islet yield by eliminating the purification step and grafting pancreatic dispersions. Mirkovitch and Campiche (1976) ameliorated diabetes in depancreatized dogs by intrasplenic autografts of a collagenase treated pancreatic dispersion. Kretschmer et al (1977) observed similar results. While the dogs exhibited fasting normoglycemia, the k value during glucose tolerance tests was subnormal. Although optimal results were not observed with the dispersed pancreas model, it was possible to examine some of the technical aspects of transplantation that had been previously addressed in rodents in a large animal model.

Only recently have investigators been able to isolate a sufficient number of islets to attempt islet cell transplantation in dogs. Horaguchi and Merrel (1981) used a ductal perfusion of collagenase to increase the islet yield, and observed three of five dogs receiving islets into the liver and two of two dogs receiving islets to the spleen were rendered normoglycemic. Warnock et al (1983) were able to render totally pancreatectomized dogs normoglycemic through splenic reflux of isolated pancreatic fragments using a modification of the Horaguchi and Merrel technique. However, the recipients exhibited diminished k values to intravenous glucose tolerance tests. Using similar methods, islets are now being isolated from human cadaveric pancreases (Rajotte et al 1987) with the isolated islets being cryopreserved and "banked" (Kneteman and Rajotte 1986) in preparation for transplantation in humans. Current research is directed at increasing isolation yield and solving immunologic obstacles with successful clinical islet cell transplantation as the ultimate goal.

APPENDIX 2
RAW DATA
CONTROL DOGS

DOG#	TIME	HR	HCT	IRI	CP	IRG	PG	EPI	NE	LAC
G309	-30	72	40.0	3.3	0.050	172	82	31	83	0.990
	-15	72	40.0	5.8	0.075	172	82	52	117	0.828
	0	0	0.0	0.0	0.000	0	0	0	0	0
	15	132	43.0	2.6	0.050	185	68	63	125	1.027
	30	138	43.0	3.0	0.050	193	71	199	417	1.107
	45	132	44.0	2.7	0.050	229	69	136	267	1.187
	60	150	0.0	5.5	0.050	300	73	136	642	1.119
	75	90	43.0	6.8	0.143	221	78	163	325	1.135
	90	96	41.0	4.9	0.079	244	75	73	317	0.943
	90	102	47.0	13.9	0.208	140	95	0	426	0.903
G205	-15	126	45.0	13.3	0.233	132	107	0	333	0.891
	0	132	45.0	7.2	0.132	144	99	0	434	0.943
	15	186	46.0	3.1	0.059	159	84	0	582	1.187
	30	186	47.0	4.2	0.086	189	91	0	709	1.301
	45	186	47.0	5.4	0.084	230	87	0	678	1.473
	60	180	50.0	5.5	0.144	247	91	0	757	2.397
	75	162	45.0	3.8	0.098	160	96	0	441	2.145
	90	168	42.5	5.4	0.114	159	108	0	205	1.438
	-30	102	46.0	6.4	0.119	165	91	208	129	1.105
	-15	108	46.0	8.6	0.159	169	91	104	678	0.735
G183	0	90	46.0	8.2	0.152	184	94	254	218	1.042
	15	174	47.0	12.7	0.249	177	91	381	485	1.529
	30	180	48.0	8.0	0.183	208	86	427	428	2.625
	45	180	49.0	7.8	0.226	299	84	462	1398	2.854
	60	180	49.0	12.6	0.276	426	83	265	453	2.888
	75	114	46.0	3.5	0.102	223	71	192	377	1.929
	90	96	45.0	3.9	0.000	0	88	67	234	1.119
	-30	96	48.0	4.0	0.062	100	99	127	503	1.019
	-15	72	46.0	7.0	0.088	77	100	253	646	0.845
	0	78	46.5	8.0	0.138	83	96	563	513	1.059
G463	15	186	47.5	4.7	0.086	93	90	380	626	0.810
	30	192	46.0	6.3	0.101	124	89	338	851	1.450
	45	204	46.5	4.9	0.101	146	86	436	1385	2.237
	60	198	48.0	4.5	0.088	146	89	506	1262	2.899
	75	102	47.5	2.8	0.056	147	89	253	779	2.043
	90	84	45.5	2.5	0.056	112	94	492	708	1.438
	-30	84	49.0	6.5	0.087	100	87	283	236	0.862
	-15	84	48.5	5.5	0.108	102	84	397	295	0.868
	0	96	49.0	15.7	0.271	131	91	176	967	0.972
	15	168	49.5	4.5	0.103	125	82	307	303	1.153
G411	30	180	48.0	4.6	0.088	140	81	500	670	1.495
	45	192	49.0	5.5	0.124	163	87	220	626	1.826
	60	198	50.5	10.5	0.229	197	91	293	221	3.345
	75	120	47.0	3.7	0.113	155	77	308	267	3.306
	90	96	46.5	4.0	0.116	138	81	345	254	2.180
	-30	96	43.0	4.3	0.081	103	84	31	323	0.914
	-15	102	40.5	6.0	0.081	113	86	42	344	1.077
	0	78	41.0	7.3	0.106	108	86	63	323	0.990
	15	0	44.5	5.0	0.124	177	77	115	438	1.495
	30	0	45.5	9.0	0.222	202	79	147	488	1.758
G403	45	0	45.5	6.2	0.218	244	77	0	0	2.275
	60	0	46.0	7.0	0.188	314	72	241	696	2.249
	75	0	43.0	3.4	0.083	153	62	63	1112	1.495
	90	0	41.0	4.2	0.082	137	84	147	660	0.879

TRANSPLANT DOGS

F187	-30	84	40.0	7.0	0.067	116	91	113	515	0.573
	-15	72	41.0	3.7	0.053	110	91	188	462	0.805
	0	0	41.0	6.1	0.066	113	92	113	308	0.741
	15	168	44.0	4.2	0.050	118	79	213	477	0.000
	30	168	45.0	4.6	0.050	146	79	325	715	0.000
	45	168	46.0	10.8	0.099	267	82	488	800	1.644
	60	168	50.0	8.5	0.097	408	84	900	1046	2.580
	75	126	43.0	3.6	0.069	170	70	413	738	2.488
	90	120	41.0	6.0	0.071	137	79	275	500	1.141
F110	-30	102	44.0	5.1	0.060	116	98	102	283	0.891
	-15	84	41.0	4.9	0.064	117	98	31	110	0.874
	0	108	42.0	5.2	0.056	122	98	133	119	0.816
	15	180	45.0	4.4	0.050	156	90	235	594	1.136
	30	186	47.0	11.7	0.146	251	97	387	347	1.587
	45	186	47.0	6.2	0.086	308	97	430	302	2.020
	60	186	48.0	7.0	0.065	438	90	1268	1243	2.751
	75	120	44.0	6.0	0.089	242	79	440	219	2.854
	90	102	44.0	2.3	0.050	171	78	389	155	1.313
H292	-30	108	46.0	7.1	0.091	215	105	109	152	0.666
	-15	80	47.0	6.6	0.097	206	107	68	344	0.770
	0	80	45.0	6.8	0.093	189	108	95	408	0.573
	15	0	55.0	4.2	0.065	226	85	212	640	1.290
	30	0	55.5	10.9	0.095	451	85	636	596	2.334
	45	0	55.0	7.8	0.101	630	87	2000	978	3.322
	60	0	55.0	7.8	0.077	792	83	1062	682	4.555
	75	144	51.5	3.6	0.050	334	68	450	456	4.223
	90	0	51.0	1.8	0.050	302	73	439	436	2.214
F199	-30	96	42.0	6.8	0.062	117	100	59	219	1.273
	-15	78	42.0	8.3	0.100	143	108	166	166	1.100
	0	78	42.0	8.1	0.098	142	105	213	157	1.227
	15	186	45.0	6.4	0.087	154	96	83	1180	2.237
	30	192	47.0	12.9	0.142	211	100	293	257	4.001
	45	192	49.0	14.7	0.150	264	96	163	361	4.246
	60	186	50.0	9.4	0.117	312	91	135	362	5.502
	75	120	46.0	4.7	0.075	215	83	15	142	4.749
	90	120	45.0	4.5	0.055	178	89	0	166	3.733
F215	-30	78	37.0	5.1	0.103	114	95	131	476	0.596
	-15	78	36.0	4.2	0.100	123	87	131	525	0.642
	0	60	35.5	4.5	0.099	118	77	131	647	0.608
	15	162	39.0	3.5	0.082	118	71	276	1063	0.788
	30	162	39.5	6.1	0.104	142	67	494	2247	0.822
	45	168	40.0	4.2	0.077	205	66	305	1002	0.856
	60	162	41.0	3.2	0.092	239	68	184	1649	1.221
	75	80	39.5	3.6	0.082	172	65	29	794	0.936
	90	102	40.0	3.0	0.071	128	77	218	611	1.062
F146	-30	120	45.5	3.9	0.093	180	88	154	236	1.007
	-15	126	41.0	5.4	0.081	151	85	257	464	0.700
	0	120	43.0	13.7	0.079	160	87	231	591	0.839
	15	192	43.5	2.3	0.065	150	66	296	391	0.936
	30	204	45.0	3.8	0.077	169	78	180	300	0.970
	45	204	45.5	3.9	0.081	202	73	347	536	1.267
	60	222	45.5	3.7	0.083	244	65	489	764	1.952
	75	120	40.0	3.5	0.079	186	67	180	391	0.902
	90	120	39.5	2.3	0.067	148	72	129	418	0.628